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SULPHUR BACTERIA

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SULPHUR BACTERIA

A MONOGRAPH

BY

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PREFACE.

In the last two decades considerable progress has been made in every branch of Bacteriology; and of the different branches, that dealing with the sulphur bacteria has received much attention from foreign investigators. An attempt is here made to present to English readers the present position of our knowledge of the sulphur bacteria.

The researches on these organisms are spread over a wide field of investigators, and are described in divers languages. As for the most part such studies have been confined to partial aspects of the subject, there should be room for a book such as the present, which aims at summing up the whole position. The only section which is scantily treated is that of Ecology, and the reason for this is that this subject is very adequately dealt with in Bavendamm's *Die farblosen und roten Schwefelbakterien.*

The study of the sulphur bacteria supplies matters of interest not only to the bacteriologist and the botanist, but also to the chemist who is interested in the analysis of water, in the preservation of the purity of water reservoirs, and in the chemical changes which take place in sewage-laden waters.

These bacteria form a group of peculiar interest to the botanist because of the possible relationship of many of their species to the animal kingdom. Their study throws considerable light on the structure, methods of reproduction, and physiological activities of organisms that stand on the borderline between plants and animals; and this work has been

primarily written as a contribution to botanical literature. The author's claim to make the effort rests on a study of the sulphur bacteria over a period of fourteen years. Some of the work has already appeared in various scientific periodicals, but a large portion is here published for the first time.

On the morphological side of the subject all the known forms are sketched and described with sufficient fullness to secure identification. A scheme of classification is drawn up which avoids the defects of its predecessors. Numerous schemes of classification of the sulphur bacteria have appeared, but, with one or two exceptions, these are parts of a general scheme embracing the whole of the bacteria, and have not followed a special study of the sulphur bacteria. As a consequence the later schemes have merely perpetuated the defects of the older classifications.

There are many gaps in our knowledge of the physiology of the sulphur bacteria which cannot be adequately filled by the biologist, and which await the attention of the biochemist. It is hoped that the present work will form a basis for investigations on biochemical lines.

I desire to acknowledge my especial indebtedness to my colleagues, Mr. W. G. Burrell, M.A., Dr. J. A. Cranston, Mr. J. Muil Leitch, B.Sc., and Dr. Blodwen Lloyd, for their assistance in the preparation of the manuscript and in the revision of the proofs; and to Mr. Leitch for the preparation of the Indices.

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CHAPTER I.

INTRODUCTION—GEOGRAPHICAL DISTRIBUTION—THE SULPHUR CYCLE IN NATURE—METHODS OF INVESTIGATION—PLEOMORPHISM.

Introduction.

The term Sulphur Bacteria is usually applied to the members of the group which have sulphur globules in their cells. As a result of their activities compounds of the highest importance to the higher green plants are formed ; in fact these organisms constitute a cog in the machinery of life, a breakdown in which would sooner or later result in the elimination of mankind and the higher animals.

It is inevitable that a great diversity of form should characterize a division of plants the members of which owe their grouping to a physiological trait that does not necessarily connote the possession of any other character, either morphological or physiological. An examination of the sulphur bacteria supplies sufficient evidence of the unsuitability of physiological classifications to express phylogenetic relationships. The sulphur bacteria include almost every variety of size and form that is to be found among the bacteria and allied organisms. Thus there are representatives of the bacillus, the coccus and the spirillum ; and in addition there are ovoid, filamentous and other shaped forms in the group. The size varies from one that is barely visible under the microscope to one that is large enough to permit of microtome sections.

If the bacteria as a group were eliminated from our classifications and a distribution of the sulphur bacteria among the other classes became necessary, some would be added to

the list of Algæ, others to the lower fungal groups, whilst a number would find a place among classes of the animal kingdom. If one compares, for example, the diversity of form and mode of life of *Chromatium*, *Thiobacillus*, *Thiospirillum*, and *Beggiatoa*, the phylogenetic unsoundness of the grouping becomes manifest. In the present stage of our knowledge, however, the grouping must remain in spite of this defect on account of its convenience for the further investigation of these interesting organisms.

It is doubtful whether any group of plants excels the sulphur bacteria in the numerous aspects of biological interest that they show. We have in them the best examples of pleomorphism shown by any group of organisms; some of the members hold the first place in their sensitiveness to light. The sulphur metabolism is a subject of transcending interest. In addition, the sulphur bacteria show a great variety in form, in internal structure, and in the different phases of their development. If we regard these plants from the point of view of their more immediate utilitarian aspect there is much that calls for attention. In the first place, as the substance consumed by them is the foul-smelling sulphuretted hydrogen, and as the chief end product in their metabolism is the compound, namely the sulphate, which is absorbed by the higher green plants, their place in the economy of nature is obvious. The higher green plants, from which, either directly or indirectly, all human beings draw their food, are supplied with a necessary food material.

Whilst the ultimate result of their activities is productive of great benefit to mankind their more immediate effects may be highly inconvenient, for when growth takes place in water conduits or in stagnant pools, a large volume of solid organic matter is formed which chokes up the conduit pipes, and renders the water unfit for human consumption. Also, instances are known in which water thus affected has been rendered unfit for use in various industries.

Indirectly the operations of the sulphur bacteria influence the play of other forces of great economic importance. The rôle of bacteria in the transformation of muds, sands, and other

accumulations that contain organic matter, is one that is important, and is in some instances considerably influenced by the activities of the sulphur bacteria. The stench of black muds on the foreshore and the pollution which attends the heaping together of mud, sand, and organic matter on our shores cannot be neglected. Among the factors which must be reckoned with are the activities of the sulphur bacteria. Again, some of these organisms develop freely in sewage-contaminated water, and may be utilized in the detection of sewage contamination in water supposed to be sewage free. The sulphur bacteria abound in sulphur wells, and although they do not produce the salts which give such wells their real or alleged healing qualities, inasmuch as they multiply freely in such waters, the nature of the products of their metabolism is of interest to those who consume the water.

Connotation of the Term "Sulphur Bacteria."

The list of bacteria that effect changes in sulphur compounds is a fairly comprehensive one. The changes include oxidation processes as well as reduction processes. We may represent the nature of the changes as follows :—

$$\text{Sulphide} \rightleftharpoons \text{Sulphur} \rightleftharpoons \text{Sulphate}.$$

Thus complete oxidation as well as complete reduction of the sulphur element may be effected. The term sulphur bacteria embraces only those organisms which oxidize the H_2S to S, store the latter temporarily in their bodies, and then oxidize it to SO_4. The term is thus used in a restricted sense, for there are other bacteria which bring about chemical changes in sulphur compounds. Thus we have :—

1. *Thionic Acid Bacteria* or *Thiosulphate Bacteria*, which oxidize thiosulphates to tetrathiosulphates and sulphates in accordance with the equations

$$3Na_2S_2O_3 + 5O = 2Na_2SO_4 + Na_2S_4O_6,$$
$$Na_2S_2O_3 + O = Na_2SO_4 + S.$$

2. *Denitrifying Thiosulphate Bacteria*, which effect the oxidation of sulphites, thiosulphates, sulphuretted hydrogen,

or sulphur to sulphates, the necessary oxygen being obtained by the reduction of nitrates.

3. *Sulphate-reducing Bacteria*, which effect the reduction of sulphates, sulphites, and thiosulphates to sulphuretted hydrogen.

4. *Saprophytic Bacteria*, which by their activities liberate sulphuretted hydrogen from the organic molecule.

Although these four classes of bacteria are concerned with the sulphur metabolism, they are not termed sulphur bacteria.

Subdivision of the Sulphur Bacteria.—The first division is based on the presence or absence of colour.

1. Colourless sulphur bacteria.
2. Coloured sulphur bacteria.

The colouring matter is composite in structure, and the sum total of the ingredients imparts to the organism a purple colour. The purple colouring matter is also found in bacteria other than the sulphur bacteria. Whilst it has not yet been proved that the association of this colouring matter with the sulphur metabolism is other than accidental, it is a noteworthy fact that all the coloured sulphur bacteria are coloured with the same group of pigments. Although there are considerable differences in the tints of the various growths of sulphur bacteria, they differ only in degree, not in kind. The differences are due to variations in the proportions in which the constituent colouring substances are present.

Geographical Distribution of the Sulphur Bacteria.

If we consider the conditions which favour the multiplication of the sulphur bacteria, it is not surprising that their distribution is universal. They thrive equally well in salt water and fresh water. They occur wherever saprophytic decomposition results in the production of sulphuretted hydrogen, and wherever, in addition, the water is shallow, the supply of oxygen not abundant, and the temperature suitable. As these conditions are obtainable in all parts of the world, with the exception of certain areas with special conditions (polar regions, deserts, etc.), the sulphur bacteria are universally

distributed. They are noticeable in the pools among the rocks on the shore where seaweed is undergoing decomposition, in shallow pools polluted with sewage, at the mouths of sulphur wells, and in many shallow waters, both marine and fresh, in which organic matter is present in solution, and in which the conditions as to oxygen, hydrogen sulphide, etc., are suitable.

If various putrescent organic masses (remains of animals and plants) be enclosed in bottles, covered with water, and stoppered up, and if these be left exposed to light, the development of purple sulphur bacteria may almost with certainty be observed in some of them after a few weeks.

Their development on a large scale is seen in shallow land-locked bays in which seaweeds and other plant debris are in a state of putrefaction. Near Copenhagen, where such conditions prevail, and where some of the bays on their shore side are filled with decomposing material, the purple sulphur bacteria impart their colour to the whole of the water occupied by the decomposing material.

The same phenomenon has been observed in various bays in Jamaica, where broad stretches of water off certain of the shores are tinted purple from the same cause. Isolated patches of purple are comparatively common on most shores on which seaweed is undergoing decomposition. It is not surprising that observations of the occurrence of the coloured sulphur bacteria should date from early times, or that they have been recorded from many sources, for their appearance in mass is sufficiently distinctive to draw the attention of even the least observant. The record of the occurrence of the colourless sulphur bacteria is not so extensive, owing to the drabness of the colour of their mass cultures. These usually assume a grey felted appearance. Thienemann records that Aristotle remarked on the white patches in certain rivers, and that Pliny called attention to the blood-red colour of the "Vulsinischen" Sea * in the year 208 B.C. It is doubtful, however, whether the sulphur bacteria were responsible in these cases,

* Now called Lago di Bolsena, in Etruria, Italy.

for the white patches in rivers are almost certainly the result of the multiplication of those species designated somewhat loosely as sewage-fungi; and the blood-red water could not have been tinted by the sulphur bacteria, for these are found only in shallow waters.

The observations on coloured sulphur bacteria are numerous. The following recorders among others may be noted :—

(*a*) *Egypt, Arabia, Siberia.*—Ehrenberg, Chr. G. (1830); Hirsch, B. (1874).

(*b*) *Germany.*—Ehrenberg, Chr. G. (1830); Corda, A. J. C. (1835); Cohn, F. (1875); Zopf, W. (1882); Engler, A. (1882); Winogradsky, S. (1887); Zacharias, O. (1903); Kolkwitz, R. (1914); Düggeli, M. (1917); Buder, J. (1919); Gicklhorn, J. (1921).

(*c*) *Great Britain.*—Lankester, Ray (1873); Klein, E. (1875); Ewart, A. J. (1897); Skene, Macgregor (1914); Ellis, D. (1924-30)

(*d*) *France.*—Fontan, A., and Joly, N. (1844); Gérardin (1873).

(*e*) *Russia.*—Weisse, J. F. (1845); Winogradsky, S. (1884); Jegunow, M. (1898); Nadson, G. A. (1903); Omelianski, W. (1904); Arzichowsky, W. (1902); Elenkin, A. A. (1914); Issatchenko, B. L. (1913-14).

(*f*) *Italy.*—Trevisan, V. (1842); Hinze, G. (1914-15).

(*g*) *Denmark.*—Warming, E. (1875).

(*h*) *Japan.*—Miyoshi, M. (1897).

(*i*) *Holland.*—Beijerinck, M. W. (1904).

(*j*) *Ceylon.*—Crow, W. B. (1923).

(*k*) *United States of America.*—Waksman, S. A. (1922); Baas-Becking, L. G. M. (1924).

(*l*) *Sweden.*—Gertz, O., and Naumann, E. (1916).

(*m*) *Poland.*—Szafer, W. (1910); Strzeszewski, B. (1913).

(*n*) *Pyrenees.*—Joly, N. (1882).

It will be evident from this list that the countries represented in it are those that contain investigators, and that absence of representation is due to the lack of investigators, not to the absence of the sulphur bacteria.

Strzeszewski (1) has made a study of the plant associations

in the flora of three sulphur wells in West Galicia. In these the following grouping prevails :—

1. First Zone.—Rich in H_2S ; yellow-green *Cyanophyceæ* and purple bacteria, particularly in the case of the latter, the more motile forms. Absence of *Beggiatoa*, *Diatomaceæ*, and *Chlorophyceæ*.

2. Second Zone.—Less rich in H_2S (0·4 gram per 10 kilogram H_2O). Fewer of *Cyanophyceæ*, a few *Diatomaceæ*, and no *Beggiatoa*.

3. Third Zone.—Very small amount of H_2S. Rich in *Diatomaceæ*, *Beggiatoaceæ*, *Chlorophyceæ* and non-thermophilous *Cyanophyceæ*. Complete absence of purple bacteria.

The Sulphur Cycle in Nature.

Plant and animal remains are attacked by hordes of saprophytic bacteria, and, after death, the protoplasmic molecule undergoes a number of transformations. Such a molecule may be compared to an accumulator as a storehouse of energy. The bacteria which nature uses as scavengers liberate some of the stored up energy in the molecule, and at the same time obtain material for their sustenance. In essentials the protoplasmic molecule is composed of a complex of carbon, hydrogen, oxygen, nitrogen, sulphur, and phosphorus atoms. With each transformation the level of energy is brought lower, and the molecule becomes less complex in structure. The final stage is reached when the original component atoms of the protoplasmic molecule have become changed to carbon dioxide, water, nitrates, sulphates, and phosphates. After complete oxidation the protoplasmic molecule has descended to its nadir position as a source of energy.

Sulphur is liberated as a constituent of the amino-acids cystine and the associated cysteine, and sulphuretted hydrogen is formed, either directly or indirectly, from one or other of these compounds. This sulphide is then absorbed by the sulphur bacteria, and the sulphur ultimately appears in the surrounding water in the form of sulphates. The development of sulphates is not, however, a simple progression, for

usually there are many kinds of different bacteria in competition and some of them effect oxidation, whilst others effect the reduction of the sulphur element. Also the environmental conditions are continually changing, so that at every change the organisms which for the moment had gained the upper hand in the decomposition of the protoplasmic molecule now find themselves supplanted by other microbes which in their turn must give way to others with fresh changes in the environment.

The sulphate may be reduced to elementary sulphur if the supply of oxygen be scanty, or possibly even to sulphuretted hydrogen if there be no oxygen present. The elementary sulphur may unite with iron or other metals to form sulphides. Again, the sulphide may be replaced by carbonic acid to form carbonates. Some bacteria oxidize thiosulphates to thiopersulphates. Ultimately, however, all the sulphur is combined in the form of sulphates, in which condition it cannot be further exploited as a source of energy by bacteria. To return to our metaphor, in the condition of sulphate the accumulator is discharged, and must be connected up with some source of energy if the sulphur is to be made once more available to build up the protoplasmic molecule. This is supplied by the sun through the medium of green plants. The sulphates are absorbed by the roots of green plants, and enter into combination with the carbohydrates built up by the carbon-assimilation of the plants. Eventually the sulphur which has been caught in this stream becomes a part of the protoplasmic molecule once more, and the cycle is complete.

As the energy of the universe is derived from green plants (and a small number of minute animals), and as all animals are, directly or indirectly, nourished by green plants, the sulphur in the protoplasmic molecule of such plants may pass into and become incorporated with the protoplasmic molecule of various animals. In such cases the sulphur-containing proteins of the plants enter into the composition of the protoplasmic molecule of the animal. When the animal dies, the fate of the sulphur element, speaking generally, is the same

as that of the same element in the protoplasmic molecule of the vegetable.

The changes to which we have referred are concerned only with the relationships of bacteria and similar micro-organisms to the protoplasmic molecule in the economy of nature. Sulphur may of course be removed from the bowels of the earth, and may be taken up as a sulphate or a sulphide into the cycle governed by the activities of microorganisms. Or again, the sulphur may lie dormant in such substances as coal, and, after a rest of some millions of years in that form, be once more taken up into the stream of that cycle. When coal is burnt sulphur compounds are liberated into the air, or spread over the surface of the earth. The converse must also follow, namely, the removal from the cycle of sulphur compounds which may lie dormant for æons before being brought back into circulation. Speaking generally, all microorganismal and all combustion processes either directly or indirectly bring about the complete oxidation of the of the sulphur atom, and the reactions which bring about this condition are exothermic.

The sulphur bacteria are thus like all plants and animals in that they require sources of energy to make possible the various manifestations associated with life. These manifestations are necessarily in their totality exothermic processes. As will be shown in the following pages, however, it is probable that the purple sulphur bacteria are able to do constructive work in virtue of their ability to absorb energy from the sun's rays. In this they resemble the plants and animals that possess chlorophyll, and as in the case of green plants such energy is directed to the building up from simpler compounds of more complex substances which thus become storehouses of potential energy. The conversion of stored energy to what may be named " vital " energy takes place when the more complex substances revert to simpler forms, thereby liberating some of their energy. The same laws regulate the changes of energy in the animate as in the inanimate world.

Methods of Investigation.

Our knowledge of the sulphur bacteria has been built up by the application of three methods of examination, each of which has its advantages and disadvantages.

1. *Direct Observation under Natural Conditions.*—This is the best method of observing the developmental life-history of these bacteria, provided that the organisms can be recognized with certainty, and readily distinguished from all other organisms occupying the same field. The sulphur bacteria are then observed in the normal course of their development under natural conditions, and in competition with other organisms of the same class. The sulphur inclusions and the purple colour are noticeable features which make their identification easy, and facilitate the identification of the passage of an organism from one phase of development to another. When observations of the same mass-cultures are conducted at periodical intervals throughout the whole year, the course of the life-history of an organism is sometimes easy to follow. Also, in such cases it is often possible to observe in organic connection two different forms of growth which, without the observation of such connection, would be regarded as specifically distinct. The valuable observations of Zopf on both the coloured and colourless sulphur bacteria were obtained by this method, and the same applies to the published observations of the majority of the other morphologists who have studied these bacteria. This method is particularly valuable when the organism under observation is present in very large numbers, as is usually the case in mass-cultures of the sulphur bacteria.

2. *Glass Slide and Coverslip Observations.*—This method was favoured by Winogradsky (1–3), and is described in Chap. IV. In essentials it consists in placing material on a glass slide and observing it at periodic intervals, so that the same individuals are viewed after sufficient time has elapsed to enable them to advance in growth. This method is valuable when it supplies positive results, but no deductions are permissible from the negative results obtained by its use. If negative results are

obtained by this method it does not follow that the results will be invariably negative under all conditions of growth. The sulphur bacteria are exceedingly plastic, and it is not to be expected that all their capabilities will be manifested under the artificial conditions of the glass slide and coverslip observations. Under the stress of competition with other organisms in nature they sometimes exhibit a variety of forms that do not appear when they are cultivated under cloistered conditions. Hence if a certain phase of development is observed under natural conditions the value of the discovery is not nullified if the same development cannot be produced under certain other artificial conditions. With such plastic organisms special circumstances bring out special reactions. Special emphasis is laid on this point because in the observations of some of the investigators, and notably of Winogradsky, it seems to be assumed that that which does not appear under artificial conditions cannot appear under natural conditions under any circumstances whatsoever.

3. *Method of Pure Culture.*—Pure cultures of the sulphur bacteria have been obtained by Keil for the colourless, and by Bavendamm for the coloured, bacteria. This is the most valuable method of observation in the investigation of physiological problems, many of which cannot be determined in any other way. The same defect is, however, inherent in this as in the previous method, for the course of the life-history of bacteria is seldom the same under the artificial conditions under which the cultivation must necessarily be conducted, as it is under natural conditions. Hence whilst the positive results obtained by this method are invaluable, and particularly in physiological investigations, judgment must be deferred if the results are negative, especially if such results are in contradiction to the positive results obtained by other methods.

All three methods have contributed their share to the building up of the present body of our knowledge of the sulphur bacteria.

Pleomorphism.

By pleomorphism is meant the capacity of an organism to assume diverse forms. A culture of bacteria may contain several variant forms simultaneously, or these may be formed in succession. Bacteria in general are noteworthy for the ease with which changes in form occur in them. The ease with which certain physiological changes bring about changes in the external appearance of bacterial cells is a matter of common knowledge. Probably no other organisms possess greater plasticity in this respect. Unless, for example, a wide range is given to the dimensions of a bacillus, a statement of its length or its thickness has little value for purposes of identification. It is a matter of common observation, too, that, under certain circumstances the rate of growth in size and the rate of division of bacteria do not run parallel. The rate of each is dependent on circumstances of which at present we have very little knowledge. If the rate of division is accelerated the individuals of successive generations become progressively smaller. Under certain circumstances the rate of division of *Crenothrix polyspora*, one of the iron bacteria, may so far exceed the rate of its growth that the individuals may be so reduced in size that they are barely visible with the highest powers of the microscope. Again, the change that takes place in the size of the individuals of a bacterial culture after frequent subculture is well known. Further, every living bacterial cell is at all times covered by a delicate covering of slime formed by transformation of the outermost layer of the membrane. Given certain conditions, this slime formation increases in intensity, and the motility of the cell is retarded, and may even cease altogether. Conversely, by the elimination of excessive slime formation non-motile forms may be made to assume motility.* Other instances could be adduced in illustration of the morphological plasticity of bacteria. Over 2000 species of the genus bacillus

* By subculturing at the very earliest sign of growth on the surface of agar-slope cultures, the motility of organisms may be considerably enhanced, and even forms regarded as non-motile may be made to assume motility (Ellis (2-3)).

alone have been described, and, in all probability, with greater knowledge of the capacity of bacteria to accomplish morphological changes it should be possible to reduce this number considerably. Organisms like *Bacillus subtilis*, for example, must have been described under numerous names, for the aggregate of its characters is never exactly the same from one generation to another.

The difference between these slight changes in the form of bacterial organisms and pleomorphism is one of degree not of kind. The change of form in pleomorphism is more pronounced and probably more sudden in its appearance. The sulphur bacteria are at a stage in which, compared with the lower bacteria, a slight advance in evolutionary progress has taken place. Developments that are mere tendencies in the lower bacteria have become more or less crystallized in them. In some the filamentous condition is normal for the species, slime formation is a characteristic feature of development, and the zoogloea condition with its enclosed mass of bacteria makes its appearance. Such developments have not, however, resulted in the stabilization of any one of the forms of growth, and so in the life-history of some of the species it is possible to find forms of growth of different morphological features. The changes in form may take place in successive generations or the differences may all appear in the same generation. Or again, the culture may be unimorphic for several generations and then, under the influence of certain (at present unknown) changes, become pleomorphic. It is evident that the proof of the existence of pleomorphism can be obtained only by chance, or by the long-continued investigation of an organism, when at length some proof may be expected. It cannot be demonstrated with regularity, because the conditions which bring about its manifestation are at present unknown.

The evidence for the existence of pleomorphism in the sulphur bacteria may now be set forth.

Evidence of the Occurrence of Pleomorphism in the Sulphur Bacteria.

Lankester (1) and (2) found a "peach-coloured bacterium" on decomposing animal remains (caddis worms) in an Oxford laboratory. This organism (*Bacterium rubescens*) assumed a bewildering variety of forms, all tinted with a purple colouring matter which Lankester named Bacteriopurpurin, and all contained inclusions which we now know to be sulphur granules. In this case we have either to assume that a dozen or more different species of the comparatively rare sulphur-containing purple bacteria had all settled simultaneously on the caddis worms, or that all were pleomorphic variations of one or at most two or three species. Lankester chose to make the latter assumption, and gave one name to all the variants, namely, *Bacterium rubescens*.

Warming examined the purple covering on the surface of decomposing vegetable remains on the Danish coast, and there found an organism of a similar protean habit. He regarded it as another species of the same genus as that described by Lankester, and named it *Bacterium sulfuratum*. At the time (1876) the number of bacteria of all kinds which had been described was very small, and both Lankester and Warming considered that the total number of species was very limited. Indeed, Warming deduced from his experience of *Bacterium sulfuratum* that all bacteria had an unlimited capacity for changes of form, and could all with propriety be brought within the compass of a single genus. Warming was the first to make a clear pronouncement on the existence of pleomorphism, although with the limited knowledge that was then at his disposal he overstated the case. The most important part of Warming's contribution was his discovery of intermediate forms. He figures two or three hundred varieties all found in the same medium and linking up by innumerable intermediate forms the most diversely shaped varieties. Recently Bavendamm has recorded the appearance in his artificial cultures of an organism which he considers as identical with *Bacterium sulfuratum*. This was a dull red irregular rod-

shaped organism of sizes varying from 3μ to 12μ, although some extended to 58μ. As Bavendamm regards Warming's *Bacterium sulfuratum* as a composite species he was not able to place the organism, and hazarded the conjecture that it was a *Rhabdochromatium*.

Zopf, in 1882, made a distinct advance in our knowledge. He also had found an extremely variable purple-coloured microbe, which he named *Beggiatoa roseo-persicina*. Up to Zopf's time the evidence of pleomorphism had been of a purely circumstantial nature, as is, indeed, the greater part of biological evidence. He considerably strengthened the case for pleomorphism by observing the actual transition of one type of structure into another. Thus in his *Spaltpflanzen*, Tafel 15, Figs. 3*a*, 3*b*, and 4 obviously show a stage in the transition from the filamentous to the coccus form. He thus observed the development of one fundamental form from the other. Then again, he traced the passing of coccus forms into the zoogloea condition. Zopf witnessed the actual liberation of the end portion of a thread of *Beggiatoa alba* and its conversion into a spiral structure, which on liberation was observed to swim away ; in that form it was indistinguishable from a typical spirillum of the lower bacteria. If seen apart, such an organism would indubitably have been assigned to the genus spirillum. It is evident from Zopf's work that the filamentous form, the short rod, the coccus, and the spirillum may be combined in one species.

In 1888 appeared Winogradsky's *Beiträge zur Morphologie und Physiologie der Bacterien*, which has greatly influenced subsequent investigations. In this work a completely different view is presented. He starts with de Bary's dictum that a species is not determinable until its developmental history is known. The soundness of this axiom cannot of course be questioned, but there are objections to the interpretations that are given to it by Winogradsky. The implication is made in his writings that, if an organism is put under observation under a given set of circumstances all the possible developmental phases of which it is capable will be exhibited during that period. Winogradsky's method

of observation is described on page 53. The organism under observation is confined between glass slide and coverslip, and treated with H_2S at appropriate intervals (he held that the introduction of organic matter was not necessary). When under these artificial conditions the one phase of growth under observation did not develop into some different developmental phase, he concluded in effect that the transformation was not possible under any combination of circumstances under natural conditions. He held that two phases of growth belonged to one and the same species, provided that it was possible by a continuous-observation experiment to effect the transformation of the one into the other. When he failed to accomplish this, the different phases of growth were to be adjudged as belonging to different species. On the strength of the negative results obtained in this way Winogradsky discounted the positive information obtained by the earlier investigators ; and he felt justified in consequence in launching a number of new genera and species, as each phase of growth observed by him was regarded as a separate organism. Of the numerous genera and species which are recorded by him we know practically nothing of the developmental history. As we know also practically nothing of their internal structure, or their methods of reproduction, it must be held that Winogradsky's contributions have added somewhat to the difficulties of subsequent investigations into the morphology of the sulphur bacteria.

It was inevitable that the attention given to Winogradsky's investigations should have obscured the evidence for pleomorphism obtained by the earlier writers ; for the greater part new organisms have been described after observation under only one set of circumstances, and their pleomorphic possibilities have seldom been considered. It has inevitably followed that comparatively large numbers of new organisms have appeared, and have been labelled without adequate study of their structure, their developmental history, or their methods of reproduction. The consequences which have followed this attitude when applied to the classification of the sulphur bacteria have been unfortunate, and will be described in later pages.

It is of interest here to note one definite positive result in favour of pleomorphism which was obtained by Winogradsky himself. He records the growth of cells inside the slimy covering of an organism which he has named *Thiocystis violacea*. The cells which were at first globular were observed to change shape and become elliptical until the length was double the thickness. Then he observed the escape of these altered cells from their slimy covering. The slime lost its firm consistency, became swollen, and finally disappeared, thus freeing the enclosed cells, which then disappeared from the field of view in aggregated masses of varying sizes. The removal was effected by their own organs of motility which were probably cilia.

An interesting piece of evidence in favour of pleomorphism is supplied to us by Bavendamm in his study of *Chromatium Warmingii forma minus*. Although this organism has been studied by previous investigators the variation in question was not observed until Bavendamm cultivated it under new conditions, namely, the conditions of pure culture. The result was the development of buds on the cells, somewhat similar to the buds which normally appear on yeast cells. Also the buds of different cells appeared to be able to effect fusion as though preparatory to sexual reproduction. Although this must not be regarded as a definite example of pleomorphism, it does show that when the normal conditions of growth are altered the organisms readily respond by changing their structure and normal habits.

The Author's Investigations.

The detailed life-history of the organism *Thioporphyra volutans* is given in Chap. VIII. We may here, however, call attention to the evidence in favour of pleomorphism which was obtained by the investigation of this organism. The species is normally a large uni- or diplo-coccus of a purple colour, and with sulphur inclusions (Figs. 33-35). Under certain circumstances buds are formed on the cocci in great profusion, and result in the culture fluid assuming a deep purple tint.

The buds, when quite small, are abstricted from the parent organism, each usually containing a single sulphur granule (Fig. 35). They are found in the surrounding medium in aggregated masses, and are devoid of movement. Without the proof supplied by the organic connection of the buds with the parent organism they would ordinarily have been regarded as a species of *Lamprocystis*.

Although pure cultures of *Thioporphyra volutans* have not yet been obtained it is possible to intensify its growth in an artificial medium (see p. 62), which in consequence of its growth becomes tinted purple. The individuals, however, are much smaller in size. They vary from cocci of 10μ in diameter (the normal size) to small globules that are only $1\frac{3}{4}\mu$ in diameter. With sufficient assiduity it would have been possible to arrange the cocci in a descending series in which any particular form was a fraction of a μ smaller than the preceding unit, until the minimum of $1\frac{3}{4}\mu$ had been reached.

A further proof of pleomorphism was supplied by *Chromatium Linsbaueri*, which was found in a small pool of water in the Epping Forest, near London. The normal form of the organism is ovoid, and it was this type of structure that was presented in all but one of the samples that were periodically sent to the writer. In the exceptional sample, however, it was found that about 10 per cent. of the individuals were spiral-shaped. As the general structure and colour of this organism are of a very distinctive nature, and as the two forms were absolutely alike in every other respect except that of shape, there was every reason for the conclusion that both were variants of the same organism (see Fig. 30 *C*).

Although the organism *Cladothrix dichotoma* is not one of the sulphur bacteria, it is sufficiently closely related to regard evidence of pleomorphism in it as contributary evidence of pleomorphism in the sulphur bacteria. The threads of *Cladothrix dichotoma* were observed in a particular solid medium culture to be violently agitated, and to break up into spirally wound fragments of short lengths, each of which developed polar cilia. Intercalary fragments were observed to " sidestep " from the threads, assume a spiral form, and

rapidly separate themselves from the parent threads (see Ellis (5)).

Another form of pleomorphism is observed in *Crenothrix polyspora*, another of the iron bacteria. There are occasions when this organism departs from its normal structure. The cells inside the sheath which envelops them break loose and divide up into minute round fragments, and in that state continue an existence which is totally different from that normal to this organism, so much so that seen separately the two forms would not be assigned to the same species.

The evidence points to the occurrence of pleomorphism, not as a normal, or perhaps even a frequent phenomenon in the life-histories of the sulphur bacteria, but rather as one likely to occur with great frequency if certain conditions prevail, or arise with any degree of frequency. If they do not arise then it is probable that the organism remains unimorphic indefinitely. The possibility, however, of its appearance must be taken into account in framing a system of classification of the sulphur bacteria.

CHAPTER II.

THE PRODUCTION OF SULPHURETTED HYDROGEN AND ITS ASSIMILATION BY THE SULPHUR BACTERIA.

Natural Sources of Sulphuretted Hydrogen.

1. *Decomposition of Albuminous Substances.*—Animal and plant remains furnish the most important material from which sulphuretted hydrogen is formed. These are attacked by saprophytic microorganisms, and in the resulting decomposition sulphuretted hydrogen is liberated. The majority of the forty or fifty natural proteins which occur in plants and animals contain sulphur, and probably about 90 per cent. of the saprophytic organisms in the soil are able to effect the liberation of sulphuretted hydrogen from albuminous material (see p. 26).

Emil Fischer and his pupils have shown that the protein molecule is built up of amino-acids, so that the liberation of sulphuretted hydrogen will follow from the degradation of amino-acids which contain sulphur. The sole representatives of such acids are *cystine*, the associated *cysteine*, and *glutathione*, which has recently been shown by Nicolet, and Kendall and his co-workers, to be a tripeptide γ-glutamyl-cysteinyl-glycine bearing the structure

$$H_2N.CH(COOH).CH_2.CH_2.CO\,|\,NH.CH(CH_2SH).CO\,|\,NH.CH_2COOH$$

$$[H_2N.CH(COOH)CH_2.CH_2.COOH]\,[H_2N.CH(CH_2SH)COOH]\,[H_2N.CH_2.COOH]$$

Glutamic acid. *Cysteine.* *Glycine.*

Cystine is $COOH.CH(NH_2)CH_2.S.S.CH_2.CH(NH_2).COOH$.

Cysteine can be converted to cystine by oxidation.

$$2\begin{cases} CH_2—S—H \\ | \\ CH.NH_2 \\ | \\ COOH \end{cases} + O \longrightarrow \begin{matrix} CH_2—S—S—CH_2 \\ | \qquad\quad | \\ CH.NH_2 \qquad CH.NH_2 \\ | \qquad\quad | \\ COOH \qquad COOH \end{matrix} + H_2O$$

The formation of sulphuretted hydrogen from cysteine is given in the following equation :—

$$\begin{array}{l} CH_2\text{—}S\text{—}H \\ | \\ CH\,.\,NH_2 \\ | \\ COOH \end{array} + H_2 \longrightarrow \begin{array}{l} CH_3 \\ | \\ CH\,.\,NH_2 \\ | \\ COOH \end{array} + H_2S$$

or it may be indirectly formed through such intermediate compounds as

α-Thiolactic acid ($CH_3\,.\,CH\,.\,SH\,.\,COOH$),
Thioglycollic acid ($CH_2\,.\,SH\,.\,COOH$),
and Methyl mercaptan ($CH_3\,.\,SH$).

The presence of sulphuretted hydrogen in tube cultures is easily demonstrated by means of filter paper, which has been previously soaked in lead acetate solution. The paper thus treated is placed inside a tube containing a growing culture, and turns black from the formation of lead sulphide if sulphuretted hydrogen be given off. Or, the gas may be recognized by the formation of the black ferrous sulphide in the presence of iron salts. The sulphur, when dissolved by aceto-carmine, crystallizes outside the cells, where it forms typical flat octohedra (see Fig. 34*e*), or the globules may be treated with hot potassium cyanide and ferric chloride, when they turn a blood-red colour from the formation of ferric thiocyanate $Fe(CNS)_3$. The chemical tests, however, are normally not necessary because the appearance of the sulphur globules in these cells is so characteristic that they can be identified from their general appearance.

There are means of effecting the liberation of hydrogen sulphide other than from an amino-acid split off from a protein molecule. Thus the decomposition of protein matter by some of the saprophytic bacteria is attended by the formation of a scum of sulphur on the surface of the culture fluid. Such sulphur must have been derived from albuminous matter. Its union with hydrogen to form sulphuretted hydrogen is described later.

Selinsky and Brussilowsky succeeded in effecting the decomposition of a substance in which the S was combined with two carbon atoms, and such being the case, they concluded that the formation of hydrogen sulphide could have resulted only after an antecedent liberation of free sulphur; and that the hydrogen sulphide resulted from its subsequent union with hydrogen. It must be noted that this implies the union of hydrogen with sulphur at ordinary temperature. As ferments, however, cause reactions within the living body at ordinary temperatures, which, when carried out by acids, demand heat, there should be no difficulty in accepting the possibility of the union of hydrogen and sulphur at the ordinary temperature when the reaction is controlled by vital forces.

2. *Reduction of Inorganic Compounds containing Sulphur.*

(*a*) *Reduction of Sulphates.*—Beijerinck (2) found that some bacteria, cultivated under anaerobic conditions, effected the reduction of sulphates to hydrogen sulphide. Again the production of the sulphide is a common occurrence in muds and other sediments in which the supply of oxygen is small and the supply of organic matter large. One organism of this kind which was isolated was named *Bacterium hydrosulfureum.* It is pointed out by Nadson that the ability to reduce sulphate is characteristic of a large number of organisms, given suitable conditions, and that many of such bacteria, for example *Proteus vulgaris* and *Bacillus vulgaris,* are not anaerobes, so that the reduction of sulphur may take place in the presence of an abundant supply of oxygen.

Beijerinck has studied the chemistry of this reduction and states that in black mud the following changes take place:—

(1) $SO_4^{--} \longrightarrow H_2S.$
(2) $H_2S + Fe^{++} \longrightarrow FeS.$
(3) $H_2S + Fe^{+++} \longrightarrow FeS + S.$
(4) $FeS + H_2CO_3 \longrightarrow FeCO_3 + H_2S.$
(5) $H_2S \longrightarrow S.$
(6) $S \longrightarrow SO_4^{--}.$
(7) $S \longrightarrow H_2S.$

(Quoted from Baas-Becking (1).)

Hence the sulphur atom in black mud runs the gamut of changes from complete oxidation to the form of sulphate to complete reduction to the form of the sulphide. His conclusions are generally accepted although Baas-Becking points out that in the case of one of the reactions, namely, No. (4), the change is carried out in alkaline water with a considerable buffer value, for the amount of CO_2 must be considerable, which first lowers the alkalinity and then effects the change indicated in the reaction. He argues that to effect this reaction in the mud the concentration of CO_2 in the bottom of the sea overlying the black mud of the Black Sea must be at least sixty times greater than the concentration known to exist at the surface, and concludes that in such places this particular change is not possible unless there is present another source of acidity in addition to the carbon dioxide. It is highly probable that the supply of carbon dioxide is supplemented under these conditions, for such black sand contains not only acid-producing organisms but also the organic material which makes possible their multiplication.

(*b*) *Reduction of Thiosulphates.*— Holschewnikoff found that the two organisms *Vibrio hydrosulfureus* and *Bacterium hydrosulfuricum ponticum*, which he had isolated from mud at Wiesbaden, effected the change of sodium thiosulphate ($Na_2S_2O_3$) to hydrogen sulphide when cultivated in appropriate media.

(*c*) *Reduction of Sulphites.*—Beijerinck showed that the yeast plant under appropriate conditions reduces oxy-sulphur compounds to hydrogen sulphide.

Hydrogen sulphide is thus a direct or an indirect derivative of the decomposition of organic matter under the agency of various saprophytic microorganisms. Under aerobic conditions it is broken off directly from a constituent of the protein molecule. Under anaerobic or micro-aerobic conditions its formation is more indirect and is bound up with a reduction process.

3. *By Direct Union of Hydrogen and Sulphur.*—The direct union of hydrogen and sulphur may be effected by the vital activity of microorganisms. Some writers have claimed that

a definite principle, a ferment, or something of a similar nature, is secreted by certain microorganisms, and that this principle effects the union of these elements. Miquel named it a *ferment sulf-hydrique*. The use of the word 'ferment,' to denote the agent of an endothermic reaction of this nature, is contrary to the usually accepted meaning of that term. Duclaux considered that hydrogen was liberated by the organism, and that the union with sulphur took place outside the cells in the surrounding medium, not inside them, as claimed by Miquel. Beijerinck has confirmed this statement, and has cited the following experiment in justification of his view. Two flasks are filled, one with fresh water, ferrolactate, flowers of sulphur, and ditch water, and the other with the first, second, and fourth only, of these ingredients. Both are allowed to decompose by the activity of the microorganisms contained in the ditch water. It is found that ferrous sulphide is formed *in both flasks*. As the sulphide is formed in the second flask which contained no added sulphur, he concludes that in all probability the sulphide in the first flask was also formed independently of the sulphur added to it ; and hence that the union of hydrogen and sulphur is in both cases a secondary reaction which takes place outside the cell.

It has been claimed by Rey-Pailhade that the synthesis of hydrogen and sulphur is effected by certain yeasts, and that a specific substance which he named *Philothion* is secreted by the plant to effect the union. No experimental evidence for the existence of this ferment has been supplied.

The Production of Hydrogen Sulphide under Marine Conditions.

The deeper layers of marine waters containing hydrogen sulphide are completely devoid of the fauna and flora which occupy the upper layers. The Black Sea, which was investigated by the Russian Deep Sea Expedition of 1891, is a case in point. The water was found to contain the sulphide at all depths, and below 200—400 metres macroscopic organisms were completely absent. The following table gives the amount of hydrogen sulphide at the various depths :—

Depth in Metres.	Vol. H_2S in c.c. per Litre of Water.
213	0·33
427	2·22
2026	5·55
2528	6·55

The water at the sea bottom contained twenty times as much hydrogen sulphide as the surface layers.* This seems to indicate that the sulphide in the lower layers originates from the sea bottom as a result of the active decomposition of organic matter under anaerobic or micro-aerobic conditions, for it would be difficult otherwise to account for the presence of the sulphide.

The Limans.

This name is given to the shallow salt marshes at the mouths of the Dnieper which open into the Black Sea at Odessa. The bed of these marshes is composed of black mud which reeks of hydrogen sulphide, and is alkaline in reaction. On exposure to air it turns a grey colour, but becomes black once more when covered with water. Anaerobic bacteria liberating hydrogen sulphide have been isolated from this mud, and one has been identified as the *Vibrio hydrosulfureus* to which reference has already been made. It is claimed that this organism is responsible for the reduction of oxy-sulphur compounds to the substance which imparts the black colour to the mud. This is a hydrate of sulphur and iron, and forms a black colloidal covering to the other constituents of the marsh bed.

The Author's Investigation of the Black Sand of the Clyde Estuary.

In various localities on the Clyde, the sand of the shore is deep black in colour, and its formation is intimately associated with the multiplication of bacteria which produce hydrogen sulphide (see Ellis (8)). The blackness disappears

* The adjacent Sea of Marmora and the other neighbouring seas are normal in respect to their content of hydrogen sulphide.

when the sand is allowed to dry; it then assumes a grey or sometimes a golden-brown colour, similar to the normal sand in the same neighbourhood. When it is again wetted the black colour returns. The following facts have been determined:—

(1) *Number of Bacteria.*—The following figures give the "total count" of bacteria in sand selected from different parts of the Clyde:—

No. of Sample.		
1	120,000 per gram	Calculated on sand without freeing it of its water content.
2	100,000 ,, ,,	
3	10,000 ,, ,,	
4	2,916,000 ,, ,,	
5	1,000,000 ,, ,,	
6	3,000,000 ,, ,,	
7	40,000 ,, ,,	
8	400,000 ,, ,,	
9	2,000,000 ,, ,,	
10	30,000 ,, ,,	
11	600,000 ,, ,,	
*12	100,000 ,, ,,	

It was found that at least nine-tenths of these bacteria liberated hydrogen sulphide under appropriate conditions of cultivation.

(2) *Organic Matter, Total Iron, and Moisture.*—The amounts of these constituents are given as percentages in the following table:—

No. of Sample.	H_2O.	Fe.	Organic Matter.
1	19·0	0·53	2·2
2	25·6	0·56	3·0
3	29·0	0·36	1·2
4	20·4	0·43	0·7

Two other samples examined independently by workers in the author's laboratory gave 0·187 per cent. and 0·189 per cent. respectively for the total iron content.

* Subsequent counts on other samples from the same district gave the figures 600,000, 650,000, 400,000.

The sand consists of clay, fine particles of silica, and organic debris, interspersed with black particles which in most cases form a covering to the particles of silica.

(3) *The Rocks in the Clyde Drainage Area.*—To the North are the Campsie and Kilpatrick Hills, which consist almost entirely of basaltic lavas rich in magnetite, with an iron content amounting to 10—15 per cent. or more. On the South the drainage area includes the hills that extend through Renfrewshire and Lanarkshire, which are basaltic lavas. Along the course of the River Clyde the rocks are mostly Carboniferous, and include the Carboniferous Limestone Series containing coal and ironstones, and the Coal Measures in which these are also abundant. These ironstones contain $FeCO_3$, but the carboniferous coal and shale beds also contain large quantities of FeS. Finally, in the upper reaches of the Clyde, and also on the lower reaches on the north side we find the Old Red Sandstone Series, the rocks of which are composed of a sandstone with an iron oxide matrix. It is therefore natural to find a fair quantity of iron in the composition of the sands on the shore of the Clyde.

(4) *Formation of Ferrous Sulphide* (FeS).—The statements detailed above show that the conditions are favourable for the formation of ferrous sulphide. The amount of organic matter is large enough to provide ample food for the nourishment of saprophytes; there is ample moisture to permit of their development; those organisms are present in abundance which are capable of bringing about the production of hydrogen sulphide; and in addition the sand is rich in iron. It was not found possible by a simple chemical analysis to make a direct quantitative estimation of the amount of ferrous sulphide in the sand. From work done in the author's laboratory it was concluded that the black colour was entirely due to ferrous sulphide, because when the iron was extracted, and the residue, suspended in water, treated with sulphuretted hydrogen, no black colour was produced. On the other hand, a sample from which the iron had not been extracted but which had lost its black colour by drying regained its colour when sulphuretted hydrogen was passed over it.

These facts are in accord with the conclusions of Doss, who states that black muds containing abundant organic material and impregnated with colloidal hydrous ferrous sulphide occur in many localities, both in enclosed seas and in gulfs and bays connected with the open ocean. He instances the eastern Mediterranean Sea, the Black Sea, the Sea of Azov, the Caspian Sea, and the Dead Sea. Similar muds occur around Oesel Island in the Gulf of Riga, and around the mouths of the Elbe and Weser. According to Andrussow (3) the mud from the bottom of the Black Sea contains an abundance of ferrous sulphide, and this compound occurs generally in the black shore muds and sands of the Limans that border the Black Sea. Omelianski (1) states that this mud when exposed to air becomes oxidized to a grey colour. Harder regarded the black mud found on Russian coasts as ferrous sulphide, and the lighter mud as a mixture of ferrous and ferric sulphides. He did not, however, advance any proof in support of this statement. Neither was it possible to confirm his statement that the ferrous sulphide existed in a colloidal state. Even when the sand is wet and black, the black particles are large enough to be easily distinguished from the particles of silica.

(5) *Cause of Disappearance of Black Colour on Exposure to the Atmosphere.*—It is generally held that the change in colour is due to the oxidation on exposure to air of black ferrous sulphide to oxy-sulphur compounds of iron which are not black in colour. It is also believed that the iron is dissolved out of iron-bearing minerals, and is removed as ferrous carbonate, and that it is later deposited and converted into ferrous sulphide by bacteria. The author considers that the reaction between $FeCO_3$ and H_2S is reversible, and that the light or black condition is determined by whichever of the two compounds $FeCO_3$ or FeS preponderates. The reaction is as follows :—

$$FeCO_3 + H_2S \rightleftharpoons FeS + H_2CO_3.$$

Here we have a delicately balanced state of equilibrium. With the production of H_2S under moist conditions ferrous

sulphide is formed, and the sand is black. With dehydration the reaction is reversed and more $FeCO_3$ than FeS is produced ; and it is this condition that results in a lightened colour.

The Equilibrium of Hydrogen Sulphide in Water.

Sulphur bacteria grow as readily in sea water as in fresh water, since they are not affected by the degree of salinity of the water. When, however, they multiply in sea water, the multiplication takes place in a hard water in which the concentration of the hydrogen ions is low owing to the high buffer value of calcium and magnesium salts. The equilibrium of the sulphide in such a medium has been studied by Baas-Becking (1).

The H_2S will first be dissociated into hydrosulphide ions and hydrogen ions.

$$K_1[H_2S] = [H^+][HS^-].$$

First dissociation constant, $K_1 = 0{\cdot}91 \times 10^{-7}$ at 18° C.

In the second dissociation we have

$$K_2[HS^-] = [H^+][S^{--}].$$

Second dissociation constant, $K_2 = 10^{-15}$ at laboratory temperature, also

$$K\omega = [H^+][OH^-] = 10^{-14}.$$

Hence, as the sum of the positive charges is equal to the sum of the negative charges,

$$[H^+] = [HS^-] + [S^{--}] + [OH^-]$$

$$= \frac{K_1[H_2S]}{[H^+]} + \frac{K_2[HS^-]}{[H^+]} + \frac{K\omega}{[H^+]}$$

$$[H^+] = \frac{K_1[H_2S]}{[H^+]} + \frac{K_1K_2[H_2S]}{[H^+]^2} + \frac{K\omega}{[H^+]}$$

$$[H^+]^3 = K_1[H^+][H_2S] + K_1K_2[H_2S] + K\omega[H^+] \quad \ldots(1)$$

and $$[H_2S] = \frac{[H^+]^3 - K\omega[H^+]}{K_1[H^+] + K_1K_2}$$ *

* Baas-Becking's paper shows $2K_1K_2$ in place of K_1K_2, but this does not affect the conclusions.

Noting that the solubility of $H_2S = 0{\cdot}102$ when $[H^+]$ is about 10^{-6}, then, in equation (1),

the first term is of the order $10^{-7} \times 10^{-6} \times 10^{-1} = 10^{-14}$
,, second ,, ,, ,, $10^{-7} \times 10^{-15} \times 10^{-1} = 10^{-23}$
,, third ,, ,, ,, $10^{-14} \times 10^{-6} = 10^{-20}$

The second and third terms are therefore negligible in comparison with the first,

$$\therefore [H^+]^3 = K_1[H_2S][H^+]$$
$$[H^+]^2 = K_1[H_2S]$$
$$= 0{\cdot}93 \times 10^{-8}$$
$$\therefore [H^+] = 0{\cdot}96 \times 10^{-4}.$$

Baas-Becking obtains the value $H^+ = 7{\cdot}8 \times 10^{-5}$ by using a method of solution which he names the " Cardanus method " (unknown to the author) ; and he has drawn up the following table on the assumption that the metal ions are present in very low concentration ($B^+ = 10^{-5}$) :—

H^+.	H_2S.	HS^-.	S^{--}.
10^{-5}	$2{\cdot}2 \times 10^{-3}$	2×10^{-5}	2×10^{-15}
10^{-6}	$1{\cdot}2 \times 10^{-4}$	$1{\cdot}1 \times 10^{-5}$	$1{\cdot}1 \times 10^{-14}$
10^{-7}	$1{\cdot}1 \times 10^{-5}$	1×10^{-5}	1×10^{-13}
10^{-8}	1×10^{-6}	$9{\cdot}1 \times 10^{-6}$	$9{\cdot}1 \times 10^{-13}$
10^{-9}	$2{\cdot}2 \times 10^{-8}$	2×10^{-6}	2×10^{-12}

The assumption that all the sulphide ions are in attachment to the H-ion seems arbitrary, and from observations of natural waters there appears no warrant for such an assumption.

In order to present these figures graphically he calculates the logarithm of the concentrations C_H, C_{H_2S}, C_{HS^-}, C_{S--}, and, changing the sign throughout, plots the latter three against the first, which is, of course, the *p*H of the solution :—

C_H.	C_{H_2S}.	C_{HS^-}.	C_{S--}.
− 5	− 2·66	− 4·79	− 14·70
− 6	− 3·92	− 4·96	− 13·96
− 7	− 4·96	− 5·00	− 13·00
− 8	− 6·00	− 5·04	− 12·04
− 9	− 7·66	− 5·70	− 11·70

These results may be presented graphically (Fig. 1).

With these data it is possible to ascertain the conditions of equilibrium in sulphur waters in which, according to Baas-Becking the *p*H value varies from 7·6 to 8·6. At *p*H 7·6 the difference in the logarithms of the concentrations of HS^- and H_2S is 0·55, as indicated in the graph. Hence the actual ratio of concentration $\frac{HS^-}{H_2S} = 10^{0.55} \doteqdot 3{\cdot}5$.

At *p*H 8·6 this ratio $\frac{HS^-}{H_2S} = 35$. Hence in sulphur waters the HS^- concentration is 3·5—35 times as high as the H_2S concentration.

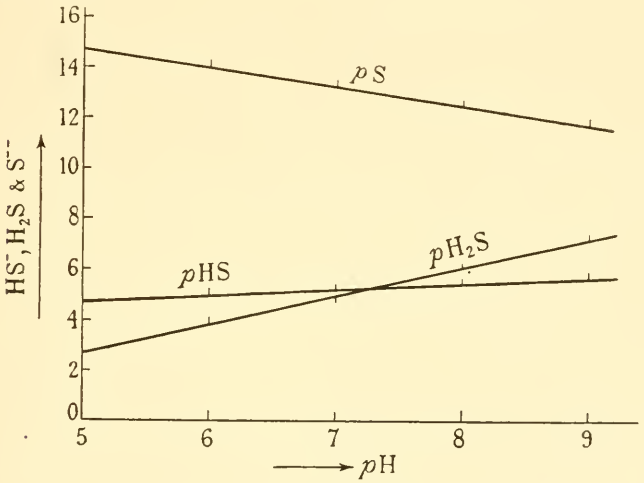


FIG. 1. (After Baas-Becking.)

(Baas-Becking appears to obtain 50—100 for the same range of *p*H values, but this difference does not affect the main argument.)

Again, at *p*H 7·6, $\frac{HS^-}{S^{--}} = 2{\cdot}5 \times 10^7$,

and at *p*H 8·6, $\frac{HS^-}{S^{--}} = 2{\cdot}35 \times 10^6$.

It therefore follows that the concentration of HS^- between *p*H 7·6 and *p*H 8·6 is somewhat greater than the concentration of H_2S, and far greater than the concentration of $[S^{--}]$. From the above facts he makes the assumption that the

sulphur bacteria absorb not the H_2S, but the hydrosulphide. He is supported in this assumption by the fact that the change from H_2S to S is from a lower to a higher level of energy. Interesting figures bearing on this matter have been published by Lewis and Randall:—

$$HS^- = S^{--} + H^+ - 20{,}470 \text{ calories.}$$
$$H_2S(aq) = HS^- + H^+ - 9470 \quad ,,$$
$$H + S(rh) = H_2S(aq) + 6490 \quad ,,$$
$$S(rh) + 2O_2 = SO_4^{--} + 176{,}500 \quad ,,$$

Baas-Becking has utilized these or similar figures to indicate the free energy levels of these compounds in the form of a scale (see Fig. 2).

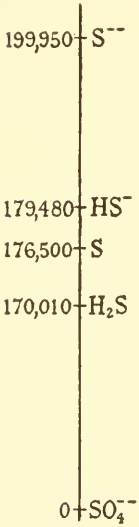


FIG. 2.

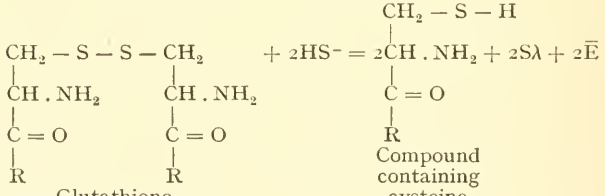
It would thus appear that the sulphur bacteria must expend rather than derive energy from the conversion of H_2S to S. For this reason it is concluded that the necessary energy is obtained by the dehydrogenation of the HS^- according to the following equation:—

$$HS^- = H^+ + S^{--}.$$

The HS^- is obtained presumably not from H_2S but in other ways, as for example by the reduction of oxy-sulphur compounds through the agency of other bacteria.

Following the scheme of oxidation suggested by Wieland and by Hopkins as taking place in yeast in the absence of oxygen, Baas-Becking suggests that the following represents the reaction by which sulphur develops in the cells of the sulphur bacteria:—

(I)*



Glutathione (contains cystine and glucosamin).

Compound containing cysteine.

$\bar{E}$ stands for electron.

* Refer to p. 20 for the most recent constitution assigned to glutathione.

Thus sulphur is produced and energy liberated. The following reactions then take place:—

(II)

$$\begin{array}{l} CH_2 . S . H \\ | \\ 2CH . NH_2 \\ | \\ C = O \\ | \\ R \end{array} + H_2CO_3 = \begin{array}{l} CH_2 - S . S . - CH_2 \\ | \\ CH . NH_2 \\ | \\ C = O \\ | \\ R \end{array} \qquad \begin{array}{l} | \\ CH . NH_2 \\ | \\ C = O \\ | \\ R \end{array} + H . CHO + H_2O_2$$

(III) $$H_2O_2 = H_2O + \tfrac{1}{2}O_2$$

Thus oxygen is developed inside the cell, which is utilized to oxidize the sulphur to the sulphate,

$$S^{--} + 2O_2 = SO_4^{--}.$$

The conclusions of this worker cannot be taken as final, for all the pieces do not "fit." Thus sulphuretted hydrogen is more soluble in an alkaline than in an acid medium, and yet in the table given above an increasing alkalinity (H^+ from 10^{-5} to 10^{-9}) is associated with a decrease in the amount of H_2S in solution (from $2{\cdot}2 \times 10^{-3}$ to $2{\cdot}2 \times 10^{-8}$). Again, Baas-Becking insists on the absence of organic matter from the beds in which the sulphur bacteria are developing, and the uselessness of such matter in the metabolism of these organisms. Under natural conditions the reactions which according to him take place in the sulphur metabolism could not occur except in the presence of an ample supply of organic matter: for all of them are the direct results of the decomposition of organic matter through the agency of saprophytic and other microorganisms. Finally, the beneficial effect of supplying the sulphur bacteria with a fluid containing sulphuretted hydrogen in solution is well established, and it is proved that the organisms assimilate this substance directly in their metabolism; and this in spite of the fact that the energy level of hydrogen sulphide is below that of sulphur. The sulphur appears to be excreted and oxidized to sulphate outside the cell. There is no reason to suppose that the sulphur takes further part in metabolism any more than it does in the metabolism of the thionic acid bacteria which deposit the sulphur outside, instead of inside the cell.

CHAPTER III.

THE METABOLISM OF THE SULPHUR BACTERIA.

The Food Requirements and Sources of Energy of the Sulphur Bacteria.

General Considerations.—The physiological status of any microbe is determined when its nitrogen and its carbon sources of supply are known. The protoplasm of the sulphur bacteria, like that of all other organisms, is built up of the elements C, H, O, N, S, P, and of these, definite provision must be made in all preparations of bacterial cultures for the supply of nitrogen and carbon. Of the other elements, hydrogen and oxygen are present in water as well as in other substances in the nutrient media, and the remainder are required in such small quantities that definite provision for their supply is not necessary. In addition, of course, the supply of mineral salts must be taken into consideration, but as the differences among bacteria in this respect are very small they play no part in making physiological distinctions.

The sources of energy of an organism are also a matter for consideration. *Autotrophic* organisms like the green plants must build up the complex protoplasmic molecule from such simple substances as carbon dioxide, mineral salts and water; and the necessary energy is obtained from the light of the sun. The presence of chlorophyll in green plants enables them to "capture" the energy contained in light, and to utilize it for their metabolic purposes. Thus *carbon-assimilation* takes place and carbohydrates are built up from the carbon dioxide and water of the atmosphere. So far as is known, the energy of the sun is not directly responsible for the further building up of carbohydrates into the still more complex proteins, the

formation of which precedes the building up of the most complex molecule of all, namely, that of living matter. As, however, there is no primary source of energy other than that of the sun, it must be presumed that more carbohydrate is formed by the green plant than is required for its immediate purposes, so that a secondary source of energy is available to effect the further synthesis of the carbohydrate into proteins by a dissociation of a part of the surplus carbohydrate into simpler substances. *Heterotrophic* plants have the advantage of being able to assimilate substances that already contain a potential source of energy, which may be utilized for the building up of protoplasm and also for the carrying out of such vital processes as reproduction, cell division, motility, etc.

All processes which originate from the vital activity of an organism are classed as *metabolic*. If they are such as result in the production of more complex substances, they are termed *anabolic;* if they result in the breaking down of substances, they are termed *katabolic*. All the vital processes in a living organism are thus to be regarded as consisting of two main streams, one leading upwards and culminating in the formation of protoplasm; and the other leading downwards and culminating in the formation of completely oxidized substances, such as nitrates, sulphates, phosphates, etc.

A closer examination of the various katabolic processes shows that the relationship of the reaction to the protoplasmic molecule is not of the same intimate nature in all cases. Three kinds of katabolic processes may be distinguished :—

1. Processes that result from the direct operation of the protoplasmic molecule itself, that is, those in which there are no special secretions for the purpose.

2. Processes that are activated by the agency of a special secretion of the protoplasm ; here the secreted material is in such intimate relationship to the protoplasm that injury to the latter results in the destruction of the former.

3. Processes that are activated by a special secretion which, unlike that of the second class, can be retained in an active condition after the organism is killed.

It is not always possible to draw sharp lines of distinction between these three classes of reactions.

Fermentation. — The history of the terms *Ferment* and *Fermentation* will give some explanation of the slight confusion that exists in the use of these words. The word ferment comes from the Latin *fervere*, to boil, and referred originally to the apparent "boiling" that took place when yeast in fermentation gave off copious bubbles of carbon dioxide. At first the *liquid* in which this bubbling took place was named the ferment, and is so named by bakers to the present day. With greater knowledge the term was restricted to the organism that caused this bubbling. With a still further advance in our knowledge the term fermentation was applied to other processes in which a medium was chemically affected by the activities of microorganisms even although there was no obvious gas production ; such, for example, as the production of lactic acid in the souring of milk. Then it was ascertained that in many cases the actual chemical work was done, not by the organism directly, but by a secretion formed from it, which in some instances could operate after the organism itself was dead. Thus arose the distinction between an *organized ferment* and an *unorganized ferment*, the former referring to the cases in which the protoplasm itself accomplished the work, and the latter to those cases in which the secretion was recognizable apart from the protoplasm producing it. The special secretion then became known as the *unorganized ferment*, or more simply as the *ferment*.

The organized ferments fall into the first of the three classes of katabolic processes cited above, and the unorganized ferments into the third.

Metabolism and Fermentation.

The view is adopted in this work that *all* fermentative processes are of a katabolic nature. Katabolic processes as a whole all come under one of two categories :—

1. Reactions which convert food substances to a form more readily assimilable by the organism.

2. Reactions which place at the disposal of the plant (or animal) a supply of energy to carry on various vital activities.

It is impossible to select a single feature which will enable us to make a distinction between the fermentative and the katabolic processes. Indeed all katabolic processes associated with vital activities may thus with propriety be named fermentative. In actual practice one of two procedures is followed in attempting a distinction :—

1. The limits are not specifically defined and only those processes are named fermentative that bear some resemblance to typical reactions such as, for example, the fermentation of sugar into alcohol by the yeast plant.
2. The term fermentation is restricted to the disruption of easily decomposable carbohydrates and similar substances where the biological significance of the process is the gain of energy to the organism.

Metabolism, Fermentation, and Respiration in the Sulphur Bacteria.

The outstanding feature in the physiology of the sulphur bacteria is the large absorption of sulphuretted hydrogen ; and the significance of this absorption is the keynote to an understanding of their physiology. The gain to the bacteria must be in terms of energy or of food. The importance of sulphur as an article of food can be dismissed, for the amount of sulphur which is absorbed is several hundreds of times greater than the amount of that substance required for the building up of the protoplasmic molecule. In this respect the sulphur bacteria do not require any more sulphur than any other bacterial organisms. Again, it has been shown that the energy level of sulphuretted hydrogen in solution is lower than that of the sulphur into which it is transformed. It is, therefore, clear that the gain to the organisms cannot be a direct one, and that the sulphuretted hydrogen must therefore participate in some larger operation which is beneficial,

and for the execution of which sulphuretted hydrogen is necessary. The salient facts may now be considered.

1. *Sulphuretted Hydrogen.*—The proof of the absorption of this substance is well established. Provided that it is not presented in too concentrated a form, large quantities are absorbed, and the optimum estimated by Bavendamm for *Lamprocystis*, namely, a concentration equal to 25 mm. pressure of Hg is probably the optimum also for the majority of the sulphur bacteria, if not for all of them. It is also established that these organisms effect *within* their cells the transformation of the sulphide into elementary sulphur, for the latter is found in quantity inside the cells. The agency of the bacteria in effecting the oxidation of the element to the sulphate is more open to question. It is true that sulphate accumulates in the neighbourhood of the cells, but more definite proof is required of bacterial agency since the oxidation of the sulphur to sulphate in an aqueous medium also takes place in the complete absence of organisms. As the sulphate in cultures of the sulphur bacteria is not found within the cells, its formation may well be due to non-vital agencies.

2. *Organic Matter.*—Winogradsky (1) and (2) came to the conclusion that the sulphur bacteria do not assimilate organic matter, and, further, that their development was hindered, if not stopped, by the presence of organic matter in more than the very smallest quantities. The second observation is obviously incorrect, since under natural conditions these forms flourish in waters which often contain very large quantities of organic matter. It is a matter of common observation that *Beggiatoa alba*, to take a prominent example, flourishes most abundantly in water contaminated with sewage matter. The present writer has found this organism in waters containing sufficient organic matter for the support of hundreds of thousands of bacteria per cubic centimetre. Again, *Thioporphyra volutans* flourishes in pools that contain in many cases saprophytic bacteria to the order of millions per cubic centimentre. It is very seldom indeed that the sulphur bacteria, under natural conditions, are found except in the presence of organic matter in quantity.

The assimilation, as distinct from the toleration of, organic matter is more of a disputed matter. Nadson inferred the assimilation of organic matter because he was able to keep sulphur bacteria in a living condition for a long time in the absence of sulphuretted hydrogen. It is difficult to see the validity of the inference. Molisch (4) showed that *Chromatium Okenii* thrives particularly well in a medium containing 1 per cent. peptone and 1 per cent. dextrin. Skene emphasizes the unimportance of organic matter, and regards it as a negligible factor in the metabolism of the sulphur bacteria. This writer does not do full justice to the facts which were discovered by him, for whilst the best growth was obtained when ammonium sulphate was the source of nitrogen he obtained appreciable growths when such sources of nitrogen as peptone, asparagin, urea, glycocoll, ammonium nitrate or calcium nitrate were used. He, however, appears to have placed a doubtful construction on these facts. Growth in presence of organic matter was regarded as due to ammonium sulphate, produced by the decomposition of these substances by saprophytic bacteria. The cultures (which were raw) were found at the close of the experiment to give almost as strong a reaction with Nessler's reagent as ammonium sulphate itself. It must be pointed out, however, that ammonium sulphate is not invariably produced by the saprophytic decomposition of these organic substances.

The two investigators who succeeded in obtaining pure cultures of the sulphur bacteria, namely, Keil (the colourless bacteria) and Bavendamm (the coloured bacteria), regarded organic matter as unessential to their development. Both considered the sulphur bacteria to be *obligate autotrophs*, that is, that under no circumstances is organic matter used as a source of nitrogen.

The following facts, however, show that organic matter may be occasionally assimilated, that is, that the organisms are *facultative* not *obligate autotrophs*:—

(1) Skene's results are not consistent with his interpretation of them, since the organic substances that were found to favour growth do not all produce ammonium sulphate

when decomposed. This applies in particular to urea and glycocoll.

(2) Undoubted increase of growth has been observed after addition of organic matter (Molisch, Nadson, Skene).

(3) Undoubted use is made of organic matter by the closely related non-sulphur purple bacteria (Molisch).

(4) Ammonium sulphate can be replaced by calcium nitrate (Nadson, Ellis), suggesting the absence of specificity.

(5) In Bavendamm's cultures the individuals did not show the same healthy growths which characterize the cultures in nature, suggesting that the growth was weak from the lack of certain ingredients which are supplied either directly or indirectly by organic matter.

(6) The same lack of robustness characterized the present writer's cultures of *Thioporphyra volutans* in a medium from which organic matter was excluded (calcium nitrate being used as the source of nitrogen).

Whilst individually each of these points is inconclusive, collectively they appear to justify the conclusion that the sulphur bacteria satisfy their nitrogen requirements in more ways than one, and that organic matter plays a subordinate rôle as a source of nitrogen. This is rendered all the more probable because

(1) They grow best in waters containing an abundant supply of organic matter.

(2) Their artificial cultures from which organic matter was excluded do not show the same robust growths which characterize their development under natural conditions.

(3) The maintenance of growth is dependent on substances that are derived from organic matter.

3. *The Source of Carbon.*—Particular attention has been paid to this aspect of nutrition by Skene and by Bavendamm. Using ammonium sulphate as the source of nitrogen, and introducing small amounts of various organic compounds, Skene failed to obtain growth with any of the carbon compounds with which he experimented. He concluded that they

hindered rather than aided growth. Bavendamm obtained the same negative results in his experiments with pure cultures, and considered that the sulphur bacteria derived their carbon from the carbonic acid of the atmosphere. He observed that the addition of carbon compounds made no difference to the growth of *Chromatium Warmingii* and of *Lamprocystis*. The question cannot, however, be held to be completely settled by the negative results of these two investigators. They have conclusively proved that the CO_2 of the atmosphere completely suffices for the carbon requirements of the sulphur bacteria, but it has still to be proved that under other circumstances these organisms are not able to make use of carbon in any other combination.

4. *Oxygen.*—The influence of oxygen on the sulphur bacteria may be considered under three heads:—

(1) The directive influence of oxygen as a chemiotactic agency in effecting the attraction or repulsion of bacteria.
(2) The possibility of this gas being given off by the purple sulphur bacteria in the same manner as green plants.
(3) The possible necessity of oxygen to the bacteria during the process of respiration.

The first and second of these questions are treated in Chap. XI. The third may now be considered.

Respiration is a function which is common to all organisms. The majority of organisms require oxygen to effect the decomposition of highly complex substances in order that the plant may benefit by the energy which is thereby liberated. Such plants constitute the majority of the members of the vegetable world, and there are only a few that respire in a different way. The two classes of plants are known respectively as *aerobes* and *anaerobes*. The anaerobes are confined to a few organisms among the bacteria and some closely related organisms. Winogradsky was the first to call attention to the reaction of the sulphur bacteria to oxygen. He had noted that in his mixed cultures the bacteria invariably retreated from the surface of growth if oxygen were present in the same

concentration as in the atmosphere, and that they throve abundantly in a medium if it was almost devoid of oxygen. When cultivated in drop cultures the bacteria retreated to the centre of the drop, and remained there so long as the oxygen pressure was not changed. Winogradsky ascertained that while oxygen at ordinary pressure was actually harmful, it was necessary in very small amounts. He did not experiment with organisms growing in media completely free from oxygen.

Nadson advanced the opinion that sulphuretted hydrogen was assimilated for protection against the poisonous effects of oxygen rather than for purposes of nourishment. He regarded the sulphur bacteria as obligate anaerobes. Molisch and Skene, like Winogradsky, considered that a small amount of oxygen was necessary, and maintained that without it the bacteria could not oxidize sulphuretted hydrogen first to sulphur and then to the sulphate. Winogradsky and Skene thought that they had found sufficient of this gas to satisfy the needs of the sulphur bacteria in the carbon assimilation of small green organisms that were always found in natural waters in the neighbourhood of the sulphur bacteria. This idea is not convincing, for the sulphur bacteria are frequently found in natural waters in which the supply of green organisms is either non-existent or is inadequate for such a purpose.

Bavendamm, working with pure cultures, found that *Lamprocystis* and *Chromatium* throve well in a medium completely devoid of oxygen, but that both these organisms developed at their best when supplied with oxygen at a pressure of 5·2 mm. of mercury.

The conclusions of Bavendamm may be taken as final for the coloured sulphur bacteria, which resemble nearly all, if not all, so-called obligate anaerobes in that, whilst they are able to grow in a medium completely devoid of oxygen, yet assimilate this gas if it be presented to them in a sufficiently dilute form.

The above remarks apply only to the relationship of the coloured forms to oxygen. Although the colourless

organisms *Thiothrix* and *Beggiatoa* were isolated by Keil in 1912 their oxygen requirements were not ascertained in pure culture experiments. They are, however, *micro-aerophilic*, as are the coloured forms, so far as may be judged from the manner of their growth and their physiological requirements.

Observations on Thioporphyra volutans.—No specific experiments have been made to determine the physiological requirements of this organism, but it was observed that its growth in various mixed cultures invariably reached its optimum when the supply of oxygen had been nearly exhausted by the multiplication of the saprophytic bacteria. It thus thrives best under micro-aerophilic conditions. On one occasion, however, it was found to be growing in abundance on wooden piles only some six inches or so below the surface in the open water of the Clyde. Hence one member of the coloured sulphur bacteria at any rate is not hindered in its growth by the presence of an abundant supply of oxygen.

It may thus be concluded that the majority of the sulphur bacteria, both coloured and colourless, are *micro-aerophilic*, but that one at least (*Thioporphyra volutans*), and probably more, thrive in highly oxygenated waters; and it is highly improbable that these do not absorb oxygen in quantity. A diversity in the oxygen requirement is to be expected of organisms that thrive under such diverse conditions as the various members of the sulphur bacteria.

5. *Mineral Matters.*—In all plants the presence of extremely small quantities of various elements, over and above those that enter into the constitution of the protoplasmic molecule, is necessary for healthy growth. Some may be required in fairly large quantities, as, for example, calcium when used to combine with the oxalic acid formed in the leaf during metabolism.

In cultivating bacteria it is usually found advantageous to add to the culture, not pure water, but a solution containing various inorganic salts in solution. The mixture which has hitherto been used with the greatest success for the sulphur

bacteria is that used by Lieske in his investigation of the iron-bacteria. This is made up as follows :—

Ammonium sulphate ($(NH_4)_2SO_4$) . .	1·50 gram.
Potassium chloride (KCl) . . .	0·05 ,,
Magnesium sulphate ($MgSO_4$) . .	0·05 ,,
Mono-hydric-potassium phosphate (K_2HPO_4)	0·05 ,,
Calcium nitrate ($Ca(NO_3)_2$) . . .	0·01 ,,
Calcium carbonate ($CaCO_3$) . . .	10·00 grams.
Distilled water	1000 c.c.

The use of the comparatively large quantities of ammonium sulphate and particularly of calcium carbonate in this mineral solution is due to the circumstance that the nitrogen in the sulphate is required for the building up of the protoplasm ; whilst the carbonate is required for purposes of neutralization.

These organisms are indifferent to common salt (NaCl).

Issatchenko (4) gives a list of sulphur bacteria that were found by him in solutions containing either NaCl or Na_2SO_4 in proportions varying from 1 to 23 per cent.

6. *Hydrogen-ion Concentration.*—The range in the *p*H values within which growth takes place must be somewhat circumscribed, for whilst the medium of growth must be alkaline the two substances that are required in greatest amounts in the metabolism of these organisms, namely, sulphuretted hydrogen and carbonic acid, are of an acid character. Hence growth is inhibited by the presence of sulphuretted hydrogen in quantity. Baas-Becking found that the optimum for the waters containing sulphur bacteria near Stanford University was 7·6—8·6 ; his figures agree with those determined by Atkins. Under natural conditions when active growth is in progress the *p*H is progressively decreased by the acid substances liberated by the saprophytic bacteria (CO_2, H_2S, organic acids, etc.). When a certain degree of acidity is reached the growth of the sulphur bacteria will cease altogether, and will recommence only when the surplus acids have once more been used up by saprophytic and other organisms.

Baas-Becking makes the erroneous statement that the sulphur bacteria occur only in hard waters, and that a high alkalinity is maintained in such waters by the large amount of calcium and magnesium present. Organisms like *Beggiatoa alba* are found in soft and hard, as well as in marine and fresh waters. Some of these bacteria thrive in waters in which the alkalinity is not maintained by the presence of carbonates and bicarbonates. The factors determining the *p*H of sulphur waters generally appear to have been determined by this writer solely from local conditions. In soft waters, under normal conditions, the *p*H of the water is kept within a certain range by the opposing nature of two classes of bacteria. On the one hand, the saprophytic and other bacteria are constantly producing acid, whilst on the other the sulphur bacteria are using up sulphuretted hydrogen and carbonic acid. Between the two opposing forces a balance is maintained which, if it is upset, results in the disappearance of the sulphur bacteria. The same conditions hold in essentials both in hard and in soft waters, the differences in respect to the growth of bacteria being one of degree, not one of kind. In artificial cultures the maintenance of an alkaline medium is often guaranteed by the addition of chalk.

Experiment with *Thioporphyra volutans*.

It was found by chemical tests that the calcium and magnesium content of pools containing a mixture of saprophytic and sulphur bacteria did not appreciably change during a period of several weeks in which active growth of both classes of bacteria was in progress. Hence it was concluded that any changes that took place in the *p*H value of the water were due to changes in the concentration of sulphuretted hydrogen and carbonic acid. The following results were obtained :—

Time in Weeks.	*p*H.
0	7·2
3	7·3
6	7·1
8	7·6
10	7·4

Although some saprophytes, as, for example, the marine denitrifying saprophyte examined by Cranston and Lloyd, maintain an alkaline reaction, the growth of a mixed group of saprophytes is practically always accompanied by a progressive development of acid. We may attribute the sharp rise in the *p*H value between the sixth and the eighth week to the consumption of the sulphuretted hydrogen (liberated by the saprophytes) by *Thioporphyra*, which during this period was observed to grow luxuriantly. The subsequent lowering after the eighth week was found to be concurrent with a revival in the growth in numbers of the saprophytes which had suffered a slight eclipse by the previous growth of the sulphur bacteria. The most vigorous growth of *Thioporphyra* occurred on the eighth week when the *p*H value was 7·6, a figure which is within the range set by Baas-Becking for the optimum growth of the sulphur bacteria.

The Biological Explanation of the Assimilation of Hydrogen Sulphide.

It has been shown that sulphuretted hydrogen does not enter the cell in the form of food, and that the change from the sulphide to sulphur does not result in a liberation of energy. It has also been shown that an undoubted gain to the bacteria follows from the assimilation of the sulphide. Hence the advantage cannot be a direct one, and must follow because the hydrogen sulphide is a factor in a wider scale of operations than its simple decomposition. It is suggested that this substance enters the cells *as an adjunct in the respiratory process*. After absorption the sulphuretted hydrogen is chemically transformed in such a manner that hydrosulphide ions are placed at the disposal of the bacteria. As this is an endothermic process the necessary energy must be supplied from the general resources of the organism, derived from other metabolic changes. Then union of the sulphide with the sulphur amino-acids takes place, resulting in the production of free sulphur and the liberation of energy. The probable changes that would then take place have been

indicated by Baas-Becking. This view implies that the hydrogen sulphide is a part of the machinery that *transfers* energy from what is presumably the less available to the more available condition. The decomposition of the hydrosulphide is to be regarded as a respiratory process comparable in its effect to the changes that follow the absorption of oxygen in aerobic plants. It is also consistent with the fact that the absorption of hydrogen sulphide is not absolutely necessary, since this substance is not the source of either the food or the energy of the sulphur bacteria. In spite of the statements of Winogradsky, Skene and Bavendamm, that the addition of sulphuretted hydrogen is indispensable to the growth of the sulphur bacteria examined by them, there are facts to show that growth may take place in the absence of this gas. Thus Molisch (3) has found that *Chromatium Okenii* may develop in a raw medium completely devoid of sulphuretted hydrogen, and Ellis (11) has shown that *Chromatium Linsbaueri* multiplies in a culture containing no trace of this substance. The sulphur bacteria are therefore able to take advantage of more than one mode of metabolism. Not only do they change their form (pleomorphism), they also change their function. The word *pleoenergism* may be coined to express this capacity for adaptation to more than one mode of life.

Pleoenergism of the Sulphur Bacteria.

There are in this group at least four different modes of obtaining food and energy.

(1) The most important is that which may be regarded as the normal mode of existence. The interaction of sulphuretted hydrogen with the products from the first stages in the decomposition of organic remains (sulphur amino-acids) supplies the energy, whilst the nitrogen and carbon are obtained from non-organic sources (ammonium compounds and carbon dioxide, respectively).

(2) Organic remains, or the products of their initial decomposition (peptones, etc.) in association with

sulphuretted hydrogen appear in certain cases to be able to supply both food and energy.

(3) Growth and multiplication may take place under the same conditions as the preceding, but without the presence of sulphuretted hydrogen.

(4) Under special cases both food and energy may be obtained without either organic matter or sulphuretted hydrogen.

In all these modes, except the fourth, the presence of organic remains is necessary, and they may be directly utilized, or they may be used after the saprophytic bacteria have effected the first stage in their decomposition, with the production of peptones, ammonium compounds, etc. In the fourth mode, the conditions are probably unnatural, and the organism does not produce normal structures. In nature the sulphur bacteria are always associated with organic matter, and whilst normally the energy of the organism is never derived directly from the organic remains before these have suffered decomposition, they probably draw their nitrogen from the decomposing remains at several stages in their decomposition of such remains (peptones, ammonium compounds, nitrates, etc.). It is also not improbable that in nature the assimilation of several kinds of nitrogenous food takes place simultaneously. There is no reason to suppose that the sulphur bacteria are wanting in adaptability, and certainly the facts do not warrant this supposition. In this respect the sulphur bacteria are not different from the majority of other bacteria. *Monoenergic* bacteria are probably not common, and the sulphur bacteria behave as do the majority.

The Denitrifying Sulphur Bacteria.—A study of the small organism* isolated by Lieske is instructive because the sulphur metabolism is combined with a process of denitrification. This organism derives its carbon from CO_2 in the form of either carbonates or bicarbonates. According to Lieske it assimi-

* This organism is not, strictly speaking, one of the sulphur bacteria because it does not store sulphur in its cells, but the general course of the metabolism is similar to that of these organisms and may appropriately be considered in this work.

lates nitrates but not nitrites, and reduces them to free nitrogen. In its sulphur metabolism hydrogen sulphide, sodium thiosulphate ($Na_2S_2O_3$), and the sodium salt of dithionic acid ($Na_2S_2O_6$) are utilized as sources of energy and changed into the sulphate.

The metabolism of this organism is interesting because it presents certain peculiar features. It is unable to utilize the energy of the sun as do the chlorophyll plants, and it has no ready-formed stores of supply as have the parasites and saprophytes. But energy must be obtained by it to build up the protoplasmic molecule from simple substances like carbon dioxide, and to carry out all the processes associated with life, namely, movement, reproduction, etc. Finally, the organism is one of the so-called obligate anaerobes, and cannot utilize oxygen when supplied to it at the normal pressure.

The place of the oxygen of the atmosphere used by aerobic plants is taken by the oxygen liberated by the reduction of the nitrate to free nitrogen. We may follow the energy changes which take place in the metabolism.

The reduction of the nitrate to the nitrite, according to figures supplied by Waksman, demands an expenditure of energy equal to 36·6 calories.

$$2HNO_3 = 2HNO_2 + O_2 \ (- 26{\cdot}6 \text{ calories}).$$

But oxygen is produced, and if utilized to break down a fermentable carbohydrate liberates 112 calories per molecule. The whole process will result in a gain on balance of

$$112 - 36{\cdot}6 = 75{\cdot}4 \text{ calories}.$$

According to the same worker the change from the nitrite to free nitrogen is an exothermic process and therefore gives a further gain of energy:—

$$2HNO_2 = N_2 + 1\tfrac{1}{2}O_2 + H_2O \ (+ 6{\cdot}8 \text{ calories}).$$

Further the $1\frac{1}{2}$ molecules of oxygen can be utilized by the organism with a gain of 112 calories per molecule. Hence

in the change from the nitrate to free nitrogen there is a total gain in energy of

$$74{\cdot}6 + (1\tfrac{1}{2} \times 112) + 6{\cdot}8 = 249{\cdot}4 \text{ calories.}$$

The sulphur denitrifying bacteria are peculiar in that they utilize the oxygen derived from the nitrate reduction to oxidize *sulphur* compounds in place of the carbohydrate oxidized by the ordinary denitrifying bacteria. The sulphur compounds (sulphuretted hydrogen, sulphur, etc.) must be regarded as fermentable substances and their oxidation as exothermic processes (or fermentation processes), which have as their biological purpose the supply of energy to the organisms. The sulphur compounds must thus be regarded not as themselves the suppliers of energy but rather as the vehicles that are made use of in the transformation of energy. As in the true sulphur bacteria they are parts of the respiratory machinery of these bacteria. It is interesting to note that Cranston and Lloyd find that the denitrifying organism examined by them often converted 100 per cent. of the nitrate supplied to it into free nitrogen, and that if this is universally true for such organisms the whole of the nitrate is used for respiratory purposes and none is used to build up the protein molecule.

Physico-chemical Speculations in regard to Organic Matter in Sulphur Waters.

There are both biological and chemical methods for the direct estimation of organic matter in water. Indirect methods must therefore be regarded as of subsidiary importance. Consideration is given to the following speculative treatment of the subject by Baas-Becking, on account of its interesting physico-chemical deductions. He attempted to prove the smallness of the organic content of the sulphur waters, rich in sulphur organisms, which are found in the neighbourhood of Stanford University, California. He bases his deductions upon the facts that the CO_2 of the atmosphere is in equilibrium with the CO_2 in the water and that the *p*H value of sulphur waters varies from *p*H 7·6 to *p*H 8·6.

The carbonic acid is dissociated in two stages :—

$$K_1(H_2CO_3) = [H^+][HCO_3^-], \quad . \quad . \quad (1)$$
$$K_1 = 3{\cdot}4 \times 10^{-7}.$$
$$K_2(HCO_3) = (H^+)(CO_3^{--}), \quad . \quad . \quad (2)$$
$$K_2 = 4{\cdot}6 \times 10^{-11}.$$

Taking into consideration the ionic product of water, we have

$$K_\omega = (H^+)(OH^-), \quad . \quad . \quad . \quad (3)$$
$$K_\omega = 8 \times 10^{-15}.$$

If we indicate the total base as (B^+) and note that the sum of the positive ions must equal the sum of the negative ions, we have

$$(B^+) + (H^+) = (OH^-) + (HCO_3^-) + (CO_3^-).^* \quad . \quad (4)$$

If we assume $(B^+) = 0$ and consider only the carbonate relationship, we have

$$(HCO_3^-) = \frac{3{\cdot}4 \times 10^{-7} \times (H_2CO_3)}{H^+},$$

$$(CO_3^{--}) = \frac{4{\cdot}6 \times 10^{-11}(HCO_3)}{H^+},$$

$$\frac{(HCO_3^-)}{(CO_3^{--})} = 2{\cdot}2 \times 10^{10} \times H^+.$$

Hence in a solution of known *p*H value we can calculate the relative proportion of bicarbonate ion and carbonate ion. Baas-Becking argues that if putrefactive bacteria were present in sulphur water the HCO_3^- would increase at the expense of the CO_3^{--}, and so alter the value of H^+. But as the *p*H value is always 7·6—8·6 this change does not take place; hence there are no putrefactive bacteria, and in consequence there is no organic matter, or not enough to make any material difference in the equilibrium of the water. It is surprising that the simple test of ascertaining directly the organic content of the water by estimating the number of saprophytic bacteria in it was not resorted to, in order to prove this conclusion. As to the actual number of bacteria that may be found in such

* Baas-Becking has inserted $2(CO_3^-)$ here, which is not correct, but this does not materially affect the equation.

waters, the reader is referred to page 64, in which the numbers found in waters containing growths of *Thioporphyra volutans* are given. Some sulphur waters contain saprophytic bacteria to the order of millions per cubic centimetre, whilst the majority of such waters contain either tens or hundreds of thousands per cubic centimetre. It is exceptional to find that the numbers are only in the order of hundreds per cubic centimetre. Even if it is held that the sulphur bacteria do not directly assimilate organic matter, it is the case nevertheless, that they must of necessity find nourishment in the products of the decomposition of organic matter, and these will be found, under natural conditions, only in waters containing organic matter. It is also remarkable that in Baas-Becking's explanation of the metabolism of the sulphur bacteria, an important rôle is ascribed to the sulphur amino-acids, and yet it is claimed that organic matter plays no part in the metabolism of the sulphur bacteria.

CHAPTER IV.

THE CULTURE OF THE SULPHUR BACTERIA.

Methods of Culture.

General Considerations.—It is desirable to cultivate the sulphur bacteria in a medium which does not contain any other microorganisms: and this is particularly the case in the investigations of physiological problems. If other organisms are present it becomes very difficult, and sometimes impossible, to estimate the exact contribution of the sulphur bacteria to the production of any particular phenomenon which may be under investigation. At the same time it is also advantageous to study the sulphur bacteria in a mixed field, for a truer presentment of their normal state is thereby given; it is very seldom that bacteria grow and multiply in nature uninfluenced by other organisms. The best conditions for the morphological study of these, as well as of other bacteria, obtain when the organism under investigation is present in its natural habitat, and is easily recognizable. Under artificial conditions, as for example in pure cultures, they are apt to depart from the normal, both in their habits and in their life histories. We must therefore accept with caution the discounting of certain *positive* results obtained from the study of the organisms under one set of conditions, on the strength of *negative* results obtained from their investigation under totally different conditions.

Winogradsky's Methods.—To Winogradsky must be given the credit for the pioneer work on methods of culture.

(*a*) *Rhizomes of Water-plants* (*Butomus, etc.*) with adhering mud were placed in a deep vessel, and water and gypsum added. The vessel, covered with a glass plate, was then placed in a

dark warm room. After some time a smell of H_2S was noticed, and a thick scum collected on the surface. The scum contained *Beggiatoa alba* and other colourless bacteria. Gypsum was added periodically to increase the growth. This method has been tried by Molisch, by Bavendamm, and by the present writer, but without success.

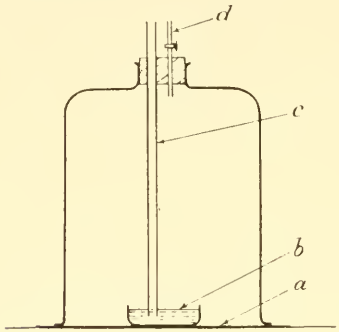


FIG. 3.

(*a*) Floor covered with damp paper on which the glass slide rests between observations.
(*b*) Small dish containing water to which 1 gram calcium sulphide has been added.
(*c*) Wide tube for occasional addition of a few drops of weak hydrochloric acid.
(*d*) Small tube to facilitate the addition of liquids down tube *c*. Winogradsky regarded the sulphur bacteria as almost completely autotrophic.* He used Strassburg tap water to which 0·005—0·010 per cent. calcium butyrate, or sodium acetate, had been added, or water from a sulphur well to which small amounts of H_2S had been added. Since he considered the sulphur bacteria to be almost completely autotrophic, he added only minute quantities of organic matter. He found the organisms to live up to nineteen days if adequately supplied with sulphuretted hydrogen, but only eight or nine days in its absence.

(*b*) *Macerated Hay and Gypsum* supplied another medium. The hay was placed in water for ten days and then boiled. The water was then decanted, a fresh supply added, and this again boiled. This process was repeated several times and the hay allowed to decompose in water. *Beggiatoa alba* appeared early.

(*c*) *Glass Slide Preparations.*—The material was placed under a coverslip, prevented from drying up, and supplied periodically with H_2S. The apparatus is shown in the accompanying diagram (Fig. 3).

(*d*) Large scale experiments carried out by method (*c*) were also successful.

His conclusions are as follows :—

1. Sulphuretted hydrogen is absorbed, oxidized first to sulphur and then to sulphate.
2. The bacteria die if cultivated without sulphuretted hydrogen.

* An *autotrophic* organism is one which is independent of organic matter in its nutrition.

3. The coloured sulphur bacteria require more sulphuretted hydrogen than the colourless forms.
4. Only a trace of organic matter is tolerated. Relatively large amounts are toxic, *e.g.* 0·1 per cent. peptone was found to be injurious.
5. The oxygen requirement is very small.
6. Exposure to light is necessary for the growth of the purple sulphur bacteria.

These conclusions are of special interest because they have formed the starting-point of later researches. Although Winogradsky states that only small quantities of organic matter are tolerated it is noteworthy that in all the experiments given above, with the exception of (*c*), the fluid in which the bacteria multiplied must have contained soluble organic matter in quantity.

The next important investigation is that of Molisch (3), who cultivated both fresh-water and marine forms. For fresh-water forms his general procedure was as follows. The material was covered with water from the river Moldau, at Prague, and included such substances as hay, boiled egg, bones, earthworms, snails, and peptone water. It was left to decompose and examined periodically for its bacterial content. For marine species such substances as Zostera (sea-wrack), a bit of crab, or fish, or some other remains, were placed in sea-water which had been brought from Trieste. In some of his cultures a layer of oil was spread over the surface to limit the supply of oxygen. Molisch obtained an abundant supply of both colourless and coloured sulphur bacteria, as well as purple coloured sulphurless bacteria. He was successful in obtaining pure cultures of the last named,* but not with the sulphur organisms. Contrary to Winogradsky, he considered that a larger amount of organic matter was necessary for the growth of these organisms. He confirmed, however, Winogradsky's conclusions that oxygen can be utilized only at diminished pressure, and that exposure to light is necessary for the coloured

* The cultivation of the sulphurless purple bacteria was first accomplished by Esmarch when he isolated *Spirillum rubrum*.

forms. For pure cultures obtained from the raw cultures mentioned above, he used the following medium :—

River water or sea water . .	1000 c.c.
Peptone	5 grams.
Dextrin or glycerine . . .	5 ,,
Agar 18 grams or gelatine 100 grams.	

Colonies were obtained by plating from this medium. He also obtained colonies by plating a drop of the inoculated melted nutrient medium under a coverslip on a glass slide and ringing the coverslip to exclude atmospheric oxygen.

Molisch's results are noteworthy in that they showed that the purple sulphurless bacteria, which are genetically allied to the purple sulphur bacteria, grow abundantly when supplied with organic matter, and in that they showed that a greater intensity could be secured in the growth of the coloured sulphur organism *Chromatium* in a medium containing organic matter but no H_2S.

A very important advance was made by Keil when he succeeded in preparing pure cultures of the colourless sulphur bacteria, *Beggiatoa* and *Thiothrix*. This investigator assumed that the following factors were necessary: sulphuretted hydrogen, oxygen, carbon dioxide, and some source of nitrogen supply, and then proceeded to show that the influences which favourably affected growth were favourable only within certain limits. The upper limit of oxygen pressure was 20 mm. Hg, the lower limit 10 mm. Hg, and the optimum 15 mm. Hg. Using a fixed oxygen supply of 15 mm. Hg pressure he determined experimentally that the optimum concentration of sulphuretted hydrogen was exerted at a pressure of 0·8 mm. Hg (about 5 c.c. H_2S in a vessel of 4500 c.c. capacity). The lower limit of carbon dioxide was 0·5 mm. Hg, approximately the amount normally present in the atmosphere. The upper limit was 360 mm. Hg, and the optimum 25 mm. Hg.

Keil then made use of these three substances in appropriate amounts to obtain pure cultures.

Pure Cultures.—(*a*) *Thiothrix.*—A clump of material made up almost wholly of *Thiothrix* threads was washed and

placed in sterilized fresh water, or a solution made up as follows :—

	Percentage.		Percentage.
$CaH_2(CO_3)_2$	0·34	$Ca_3(PO_4)_2$	0·02
$MgH_2(CO_3)_2$	0·27	KCl	0·01
$CaSO_4$	0·31	K_2S	0·01
$MgSO_4$	0·51	FeS	0·01
Na_2SO_4	0·21	CaS	0·01

A small quantity of ammonium sulphate was added, and the three gases O_2, H_2S, and CO_2 introduced.

In the second week, the threads of *Thiothrix* had begun to liberate segments from their free ends. The culture was periodically washed with sterile water. It was maintained by Keil that by this method his cultures were free from impurities because the subsequent addition to them of 0·1—0·2 per cent. of sterile peptone did not result in a development of saprophytic bacteria.

(*b*) *Beggiatoa.*—Pure cultures of *Beggiatoa* were obtained, but with greater difficulty, due to the threads being free. The purity of the cultures was tested in the same way. Keil's conclusions were :—

1. The best source of nitrogen is ammonium sulphate.
2. Organic matter, although not harmful, cannot be used as a source of N. (Contrast the conclusions of Winogradsky and Molisch.)
3. Nitrate cannot be used as a source of nitrogen.
4. Oxygen, carbon dioxide, and sulphuretted hydrogen are necessary for development, but their amounts must be within the limits given above.
5. A carbonate of one of the alkaline earths is necessary. Either the calcium or magnesium salt may be used.
6. The organism is indifferent to the salts of the alkalis.
7. A chloride furthers growth, and the culture fluid must contain phosphorus.
8. Growth increases with rise of temperatures up to 30° C. The threads rapidly degenerate above 35° C. *Beggiatoa* is somewhat more resistant than *Thiothrix.*

   Thermal death point : *Thiothrix*, 37°—38° C.
   Beggiatoa, 45° C.
9. The colourless sulphur bacteria are indifferent to light.

Jegunow (1—5) cultivated sulphur bacteria in a flat glass vessel about the length and width of an ordinary glass slide, and of a depth about a sixth of the width. Some slimy mud from the bed of one of the limans near Odessa was placed in the vessel, together with water from the same source. The vessel was exposed to air in an upright position. As oxygen was supplied from above, and hydrogen sulphide from below, the organisms took up some intermediate position, where they could obtain access to both. At the selected level they formed what Jegunow names a *Bacterial Plate*, the shape of

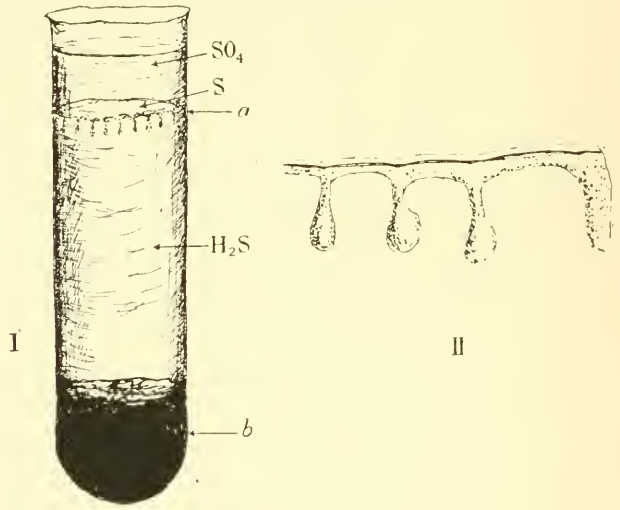


Fig. 4.

which is shown in Fig. 4 (I) at *a* and on a larger scale at Fig. 4 (II). By altering the content of hydrogen sulphide he was able at will to raise or lower the level of the bacterial plate. The chief peculiarity of this plate is the series of downward directed projections which are formed by it. The movement of the bacteria composing each projection resembles that of water in a spring. They travel down the projection, and on reaching the bottom turn round and travel up again until the top of the plate is reached. When viewed through a low-powered microscope, when the bacteria are viewed in the mass, the general appearance of a fountain is presented, which made

Jegunow bestow a second name to the aggregated bacteria, namely *Fountain Plate.*

By letting down into the fluid a very fine and weighted thread which had first been treated with very dilute ferric chloride, and then with ammonia, so that the thread was coloured a faint bluish-yellow, he was able to show some interesting relationships between the organism and the medium. Below the plate the thread turned black, following the formation of ferrous sulphide. This demonstrated the presence of hydrogen sulphide below the plate :—

$$FeCl_3 + 3NH_4OH = Fe(OH)_3 + 3NH_4Cl,$$
$$Fe(OH)_3 + H_2S = Fe_2S_3 + 6H_2O.$$

In the plate itself the bacteria were filled with globules of sulphur, whilst above the plate the colour was taken out of the thread by the action of the sulphate formed by the bacteria.

It must be stated that the phenomena described above are not of frequent occurrence, and their incidence is probably due to physical causes, in this case probably the presence of slime.

Macgregor Skene, working with raw cultures of purple sulphur bacteria, concluded that when more favourable results were obtained by the addition of certain organic compounds, this was to be attributed not directly to the organic compounds but to the ammonium sulphate liberated from these compounds by saprophytic bacteria. The most vigorous development took place in the following medium :—

Ammonium sulphate	0·75 gm.
Magnesium sulphate	0·05 ,,
Potassium dihydric phosphate	0·05 ,,
Potassium chloride	0·05 ,,
Calcium nitrate	0·01 ,,
Sodium chloride	27·00 gms.
Calcium carbonate	10·00 ,,
Distilled water	1000 c.c.

A small conical flask (50—100 c.c.) containing a depth of 1—1½ cms. of this solution was infected with sulphur bacteria, and exposed to an atmosphere of sulphuretted hydrogen. In 10—30 days a vigorous development of bacteria took place, and a rich red-purple zoogloea covered the chalk sediment.

Source of Nitrogen.—The most satisfactory of the nitrogenous compounds experimentally added was found to be ammonium sulphate, only slightly less satisfactory albumen, peptone, and asparagin. As already stated these organic compounds are not regarded by Skene as being directly assimilated. The most suitable concentration of ammonium sulphate was found to be 0·1 per cent.

Sources of Carbon.—Using ammonium sulphate as the source of nitrogen he next determined the source of carbon. A large number of organic substances were put under requisition, but Macgregor Skene came to the conclusion that not one of them was assimilated by the sulphur bacteria. Since vigorous growth occurred in the second and third generations in the mineral solution given above, he concluded that the carbon constituents necessary for the building up of bacterial cells could not have been contained in the original infecting material, and such being the case the carbon could have no origin other than the carbon dioxide of the atmosphere. Without pure cultures he was not able to confirm this statement. A series of experiments is recorded by this investigator to determine whether the sulphur bacteria obtained CO_2 from the atmosphere directly or through the medium of an autotrophic bacillus which constantly appeared in his cultures and which was easily isolated.

Eight flasks were infected with a pure culture of the bacillus and set aside for twelve days. Four were then sterilized, and to the collection four uninoculated flasks were added. Of the twelve flasks three from each series were infected with purple sulphur bacteria, so that the following experimental conditions could be obtained:—

3 flasks containing live autotrophic bacillus + CO_2 from atmosphere + sulphur bacteria.
3 flasks containing dead autotrophic bacillus + CO_2 from atmosphere + sulphur bacteria.
3 flasks containing 0 autotrophic bacillus — CO_2 from atmosphere + sulphur bacteria.
1 flask containing live autotrophic bacillus + CO_2 from atmosphere — sulphur bacteria.
1 flask containing dead autotrophic bacillus + CO_2 from atmosphere — sulphur bacteria.
1 flask containing 0 autotrophic bacillus — CO_2 from atmosphere — sulphur bacteria.

The results were inconclusive, the first nine flasks showing equal growth, and the last three, of course, none.

Tests to ascertain if H_2S *is necessary to purple sulphur bacteria.*—Flasks infected with sulphur bacteria were treated as follows :—

	Results.
1. Placed in air	0
2. Placed in H_2S gas	Strong growth
3. Usual salts added, 0·5 per cent. sodium thiosulphate + air + light	0
4. Usual salts added, 0·5 per cent. sodium thiosulphate + air — light	0
5. Usual salts added, 0·5 per cent. sodium thiosulphate — air + light	0
6. Usual salts added, 0·5 per cent. sodium thiosulphate — air — light	0
7. Sodium bisulphate, sodium sulphide, sodium thiosulphate, potassium sulphide, calcium sulphide, iron sulphide, each used in turn in place of H_2S gas	all 0

Hence he concluded that H_2S gas is a necessary addition.

Tests to ascertain the necessity of light to the purple sulphur bacteria :—

	Results. Light.	Dark.
1. Lieske's solution (see p. 44)	+	0
2. ,, ,, + dextrose, 0·15 per cent.	not recorded	0
3. ,, ,, + calcium lactate, 0·15 per cent.	,,	0
4. ,, ,, + pot. formate, 0·15 per cent.	,,	0
5. Ammonium sulphate, 0·075 per cent.; + dextrose, 0·15 per cent.	,,	0
6. ,, ,, 00·75 per cent.; + calc. lactate, 0·15 per cent.	,,	0
7. ,, ,, 0·075 per cent.; + pot. formate, 0·15 per cent.	,,	0

From which he concludes that light is necessary.

He found further that

(*a*) The purple bacteria grow better in daylight than in red light.

(*b*) The same bacteria grow better in red than in blue light.

Bavendamm repeated the cultural methods of Skene for the coloured, and of Keil for the colourless, sulphur bacteria. He also used successfully Lieske's mineral solution for the cultivation of the iron bacteria. All the culture fluids received an abundant supply of pure "calcium præcipitatum." In one instance he found that the growth of a raw culture of *Lamprocystis* obtained from a mass of decomposing Chara was so abundant that colonies of the organism rose to the surface in large numbers. By an ingenious arrangement, for details of which the reader is referred to his book, he was able to regulate the supply of oxygen and of sulphuretted hydrogen. He obtained the following results :—

1. H_2S optimum supply 25 m.m. Hg pressure.
2. O_2 ,, ,, 26 ,, ,, ,,

Hydrogen was added to raise the pressure to that of the normal atmosphere. His raw cultures were disturbed by the appearance on the surface of a thin whitish-yellow layer of sulphur in which were embedded numbers of a small colourless bacillus. By subculturing he finally obtained a growth of *Lamprocystis* which did not form a surface layer of sulphur. He satisfied himself that the culture was now pure by transferring some of it to sterile bouillon, a procedure which did not result in bacterial growth taking place in this fluid.

Experiments with Thioporphyra volutans.—A few tentative experiments have been made with this organism by the author. In nature it flourishes only in sea water containing organic matter resulting from the decomposition of seaweed, or from the presence of sewage. Both in nature and in artificial cultures active growth results only if the supply of oxygen is limited. In nature this is secured by the rapid consumption of the dissolved oxygen by the numerous aerobic saprophytic bacteria that are also present. In the laboratory

the vessels containing decomposing seaweed show a much stronger growth of the purple organism when stoppered. The growth of the organism is likewise much better in pools or in cultures in which the sulphuretted hydrogen is enough to cause a perceptible smell. Finally, active growth follows exposure to subdued light, but does not take place in the dark or when exposed to bright light. Dr. Blodwen Lloyd, in the author's laboratory, has succeeded in cultivating *Thioporphyra* in a medium made up of potassium nitrate 0·5 gram, sodium dihydric phosphate 0·25 gram, calcium malate 5 grams, and sea water 1000 cc. The nitrogen in this culture was thus obtained from the nitrate, and the carbon almost certainly from the CO_2 of the atmosphere. On further cultivation, however, the size of the organism rapidly diminished in this artificial medium, probably because the nitrate was not the best source of nitrogen. So far as the investigation has proceeded, the results confirm those obtained by Keil, Skene, and Bavendamm, but it is not yet known whether under natural conditions nitrogen is derived, as Molisch believes, from organic matter, or, as Skene thinks, from products resulting from the decomposition of organic matter.

Changes Effected in the Constitution of the Water by *Thioporphyra volutans*

The chemical changes which take place in a marine pool during the growth of *Thioporphyra volutans* have been investigated (Ellis and Stoddart). An analysis of the water of a neighbouring marine pool in which there was no decomposing matter is given for comparison in the following table :—

H_2S.		Oxygen.		Ca.	Mg.	SO_4''.	*p*H.
Grams per Litre.	c.c. per Litre.	Grams per Litre.	c.c. per Litre.	Grams per Litre.	Grams per Litre.	Grams per Litre.	
0·00032	0·21	0·013	8·89	0·20	0·315	0·408	8·1

A pool containing decomposing matter was kept under observation for ten weeks during the summer, and samples of

water from it were periodically analysed. During this period the pool, which also contained putrescent seaweed, became coloured a deep purple owing to the growth of *Thioporphyra*. Whilst the chemical changes cannot all be ascribed to *Thioporphyra volutans*, the fact that this organism during the period of investigation was far more numerous than any other indicates that it was responsible for a very large percentage of the chemical changes that took place. This applies particularly to hydrogen sulphide and the sulphates, which are respectively assimilated and secreted by this organism.

The following table gives the results of the chemical analyses :—

	H_2S.		Oxygen.		Ca.	Mg.	SO_4''.	*p*H.
	Grams per Litre.	c.c. per Litre.	Grams per Litre.	c.c. per Litre.	Grams per Litre.	Grams per Litre.	Grams per Litre.	
At commencement of investigation	·00022	·15	·015	10·5	·20	·315	·690	7·2
After 3 weeks .	·00071	·465	·016	11·4	·25	·317	·672	7·3
,, 6 ,, .	·03	19·71	·003	2·1	·126	·16	·368	7·1
,, 8 ,, .	·0072	4·7	·0028	1·96	·336	·49	1·19	7·6
,, 10 ,, .	·0045	3·0	·0018	1·26	·18	·235	·588	7·4

The results are represented graphically in Fig. 5.

In addition, a total count was made of the saprophytic bacteria, with the following results :—

Time in Weeks.	No. of Saprophytes per c.c. (approx.).	Sulphur Bacteria.
0	1,000,000	Few : objects in water not purple.
3	1,000,000	,, ,, ,, ,, ,,
6	2,000,000	Objects in water slightly purple.
8	5,000,000	,, ,, deep ,,
10	5,000,000	,, ,, ,, ,,

These tables show that for six weeks the content of hydrogen sulphide and the number of saprophytic bacteria had steadily increased, the increase in the former obviously resulting from the increase in the latter. During this period the

sulphur bacteria had also increased, and at its close had imparted a tinge of their colour to the various objects in the pool.

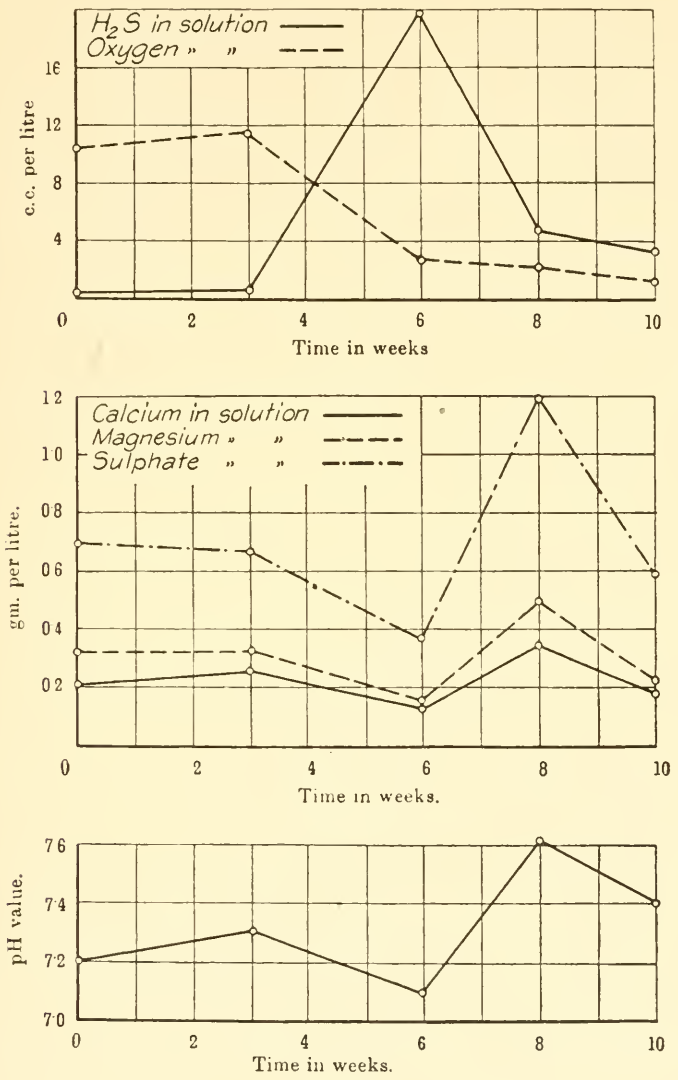


FIG. 5.

The rise in the hydrogen sulphide content is an indication that the supply by the saprophytes was greater than the

demand for it by the sulphur bacteria. After the sixth week, although there was a marked increase in the number of saprophytes, the sulphur bacteria had increased at a far greater rate, until the demand for hydrogen sulphide was greater than the supply. The gradient downwards in the sulphide graph is very steep between the sixth and the tenth weeks.

On the other hand, the downward decline of the oxygen curve is steady during the whole of the period of investigation, an indication that both the saprophytes and the sulphur bacteria were using up this element in respiration.

Whilst the graphs for hydrogen sulphide and for oxygen can be interpreted in terms of the vital activity of the sulphur bacteria, the same cannot be said of the SO_4'' graph. The indeterminate character of this graph indicates that, like the Ca and the Mg, which have similar graphs, its rise or fall does not coincide with the rise or fall in the numbers of the sulphur bacteria. This fact is consistent with the supposition that the change from S to the sulphate is not directly dependent on the vital activities of the sulphur bacteria.

The small fluctuations in the *p*H values reflect the small changes that follow the absorption of hydrogen sulphide by the sulphur bacteria, the production of other acids by the saprophytic bacteria, the partial neutralization of these acids by the calcium and other compounds, and the changes caused by evaporation or by rainfall. The results in general confirm those obtained by other investigators for other sulphur bacteria, but cannot be regarded as conclusive, because the contribution of other organisms which were necessarily present in the pool under observation could not be exactly estimated.

The growth of *Thioporphyra* reached its zenith in a medium containing enough organic matter to support 5,000,000 bacteria per cubic centimetre. Winogradsky's conclusion that the sulphur bacteria tolerate only minute quantities of organic matter is not justified. Without pure cultures it was not possible to ascertain if *Thioporphyra* assimilates organic matter directly.

CHAPTER V.

THE PRINCIPLES OF CLASSIFICATION AND THEIR APPLICATION TO THE SULPHUR BACTERIA.

The Principles of a Natural Classification, and their Application to the Grouping of the Sulphur Bacteria.

All bacteria are usually assigned to the plant kingdom, mainly because the genus Bacillus, which includes most bacteria, is, in its structure and life-history, of a distinctive plant nature. This cannot, however, be said of some of the other genera in this group. Some, as for example, *Spirillum*, are more allied to animals that to plants. In the classification of more highly evolved organisms the difficulty would be a serious one, but bacteria are at a stage of development where the distinction between a plant and an animal is not so well defined as in higher organisms. They are in fact not sufficiently far removed from ultramicroscopic organisms that cannot be assigned exclusively to either the plant or to the animal kingdom.

All those bacteria which contain sulphur inclusions are grouped under the name *sulphur bacteria*. These organisms are morphologically very varied but physiologically similar. Physiological similarity does not, however, necessarily imply genetic affinity, and the systematist is therefore confronted with a difficulty. He must effect the grouping of a large number of species on the basis of a common physiological attribute, when some of the constituent units can be assigned to the plant kingdom, whilst others readily find a place in the animal kingdom. A grouping on physiological lines is unsatisfactory, for, with increasing knowledge, it is becoming more evident that the mode of metabolism of an individual bacterium is not fixed and unalterable. As a class bacteria

are very adaptable. For example, certain bacteria lead both a saprophytic and a parasitic existence according to the nature of the habitat ; and there are denitrifying bacteria that undergo the sulphur metabolism. The insufficiency of a physiological grouping is apparent in the sulphur bacteria, for whilst the group includes almost every known variety of form and structure, the possession of sulphur inclusions does not connote any other distinction common to the whole group ; and this is the surest indication that the classification on the basis of a common physiological trait is not genetically sound. But it is nevertheless adopted in this book on account of its convenience in limiting the area of investigation. The further subdivision of the group is followed on morphological lines. All existent classifications have the defects of the classifications of higher plants in the days of Linnæus. These were based on artificial distinctions rather than on natural affinities, and to a certain extent the difficulty will always remain in the classification of lowly forms with the high degree of plasticity possessed by bacteria. Of the earlier attempts the best is that by Migula (1—3), which is based on morphological characters. This classification would have been completely successful were it not for the fact that the distinguishing features selected by him were in several instances transient and not permanent attributes.

In the classifications of Engler (1912) and of Meyer (1912), the sulphur bacteria are incorporated in a general scheme in which a few changes are made in the division of the sulphur bacteria to bring them into conformity with the general scheme adopted by the authors. There had not been during the period (1909-12) any investigations which threw any further light on the affinities of the sulphur bacteria. One addition had been made to their number, namely, *Hillhousia*, discovered by West and Griffiths in 1909.

Of the recent classifications those of the American Society of Bacteriologists are not completely satisfactory. They violate the first essential of any logical system of grouping which demands that it should not be possible to fit any organism into more than one group. Inclusion in one group

should necessarily spell exclusion from any other group, and this is not the case in the American classifications.

In the lengthy and elaborate schemes that have appeared from America there has been no attempt to question the validity of the characteristics used for the groupings. All the attributes used in earlier groupings have been taken at their face value. Those parts of Buchanan's scheme that deal with the sulphur bacteria will be here noted.

Review of the Attributes used in the Subdivision of the Sulphur Bacteria.

The chief difficulty in grouping these forms arises from the fact that hitherto so little has been done to distinguish transient from permanent characters. If the former are used for the diagnosis of bacteria, confusion inevitably follows the effort to establish the genus and species of any particular unknown organism on the basis of its morphological distinctions. The range of constant attributes among bacteria is very limited. The author has recorded (Ellis (1—3)) the results of the examination of the more important morphological characters generally used in the classification of bacteria. These are given below :—

(1) *Division in one, two, or three planes.*—It was found experimentally that a *Sarcina* which normally divides in three dimensions of space, could be made to break up into smaller units of one, two, three, or four cells. An observer remarking the cells in this last condition, would not be able to allocate them to the genus *Sarcina*, for they would not show the characteristic cell-arrangement in three dimensions of space.

In the same way species of the genus *Micrococcus*, which is distinguished by the divisions of its cells in two dimensions, were induced to break up into smaller groups varying from one to four or a small number of cells.

On the other hand, the *Streptococci*, which divide in only one dimension, do not, in the author's experience, depart from that method of division. Hence it follows that an organism showing globular cells in a culture may be a *Sarcina*, or *Micrococcus*, or a *Streptococcus*. It is possible only by an extended

investigation to ascertain to which of these genera any particular organism belongs. The author is of the opinion that no difference exists between the genera *Sarcina* and *Micrococcus*. An organism is generally ascribed to *Sarcina* if observed dividing in three dimensions, and to *Micrococcus* if in two dimensions, but it has been shown that the same organism may use both methods of division. It is an easy matter, by subculture, to effect the transformation of a *Sarcina* into a typical *Micrococcus*. Hence the number of planes of division is not constant, and is therefore not a suitable criterion for the classification of bacteria.

(2) *Motility.*—In Migula's system a distinction is made between a *Sarcina* and a *Planosarcina*, the former being non-motile, and the latter motile. A similar distinction is drawn between *Micrococcus* and *Planococcus*. Twenty-four species of *Sarcina*, *Micrococcus* and *Streptococcus* were cultivated by the author in such a way that the cells were as short a time as possible in the presence of their excretions. The result of such cultivation was invariably the same. The aggregated cells were found to break up into uni-, diplo-, tri-, and tetracocci, and at the same time motility was developed. Conversely, the aggregated stage was found to be a non-motile one; this loss of motility was correlated with an increased slime-production. It was found possible by frequent subculturing to reduce the slime formation and so to induce motility in sixteen species of *Sarcina*, five species of *Micrococcus*, three species of *Streptococcus*, and five species of *Bacterium*, all usually described as non-motile. The bacteria investigated were the following:—

Sarcina pulmonum, *aurescens*, *flavescens*, *rosea*, *flava*, *mobilis*, *fimentaria*, *gasoformans*, *striata*, *vermiformis*, *oleus*, *ventriculi*, *fuscescens*, *marginata*, and two unidentified species.

Micrococcus helvolus, *citreus*, *grossus*, and two unidentified species.

Streptococcus tyrogenus, *pallidus*, and *pyogenes*.

Bacterium hirtum, *tomentosum*, *filamentosum*, *rugosum*, and *cerinum* (Ellis (1—3)).

It is therefore highly probable that the capacity of movement is a *distinctive feature of all bacteria* under certain, at present incompletely known, conditions. It is well to bear in mind that motility is one of the fundamental properties of protoplasm, and is more readily manifested in unicellular aqueous organisms because of the obvious advantage which they obtain by its occurrence. At any rate it is certain that the fact of motility in an organism can only have a restricted use in a natural system of classification.

(3) *The Size of the Cells.*—This is an unreliable criterion for classification, as may be demonstrated by the cultivation of any one species of bacteria for an extended period, and under different conditions of growth. The production of long or short rods is often dependent upon variable external conditions, whose effect upon the organism cannot be predicted beforehand. The tendency of a normal bacillus to form long filaments under certain conditions is well known. Even the thickness of any particular species of bacteria varies. At the same time a large organism, *e.g. Bacillus megatherium*, is always large when cultivated under normal circumstances ; and others, as for example some of the nitrate bacteria, are always very small. It is therefore possible to assert that an organism is large or that it is small, but unless the given range of its measurements is a wide one, any statement of the size of an organism cannot be relied upon as a help in its identification.

Some of the sulphur bacteria show an extraordinary variation in size. This applies not only to differences in different generations, but frequently to differences in the same generation. *Bacterium sulfuratum* may show hundreds of different sizes in the same field. It is a sound rule, and one followed in this book, to ascribe to the same species all those organisms that are similar except as to size, and in which the sizes are so graded that the individuals form a series separated only by minute dimensional differences. This attitude is justified by the frequent occurrence of pleomorphism (see pp. 14 *et seq.*) in the sulphur bacteria. In the cultivation of some of the sulphur bacteria a very small change in the environmental conditions sometimes results in the production of indivi-

duals of varied size, a fact which makes it practically impossible to fix upon a certain definite size as being an attribute of any particular organism. The method adopted by Winogradsky in his study of *Beggiatoa alba* is not one which is justified in the subdivision of bacteria. When he found various sizes of that organism in the same field connected together by numerous gradations, he arbitrarily selected a particular range, and bestowed upon the individuals within that range a specific name. In this way several "species" of the genus *Beggiatoa* were established by him. Such "species" are not of the nature of "microspecies" or "elementary species" or "pure lines." The variations in size appear to be the ordinary manifestation of every culture of *Beggiatoa;* it is an aspect in the pleomorphic tendency of this species, and no more significance is to be attached to the variation than to the variation in the number of sulphur granules in the different individuals. Winogradsky established the following "species":—

Threads	up to 1μ	thick,	*Beggiatoa*	*minima.*
,,	1—2·5μ	,,	,,	*media.*
,,	2·5—4μ	,,	,,	*alba.*
,,	4—5·5μ	,,	,,	*major.*

The figures were arbitrarily chosen by him and he admits that any others would have done "weil sie nur auf Convention beruht." In other words, they are intended merely as catalogue names, and in that case it is not correct to label them as "species." Suppose a culture of this genus in, say America, shows sizes of filaments varying in thickness from, let us suppose, 1μ to 6μ, and a culture of the same genus, in say Germany, gives a crop of threads of thicknesses varying from $\frac{1}{2}\mu$ to 5μ. According to this "Convention" all the threads in both cultures between 1μ and 2·5μ must be labelled *Beggiatoa media.* Whilst there is still some ambiguity in the meaning of the term *species* all must agree that it connotes a unit that is sharply separated from other units, whether the bond of union is known or unknown. It is more reasonable to fix the name *Beggiatoa alba* (chosen for priority) for all thicknesses

of this organism, until there is evidence that there are other distinctive differences between the different classes of threads.

(4) *Cilia Insertion: Presence or Absence of Cilia.*—An investigation was instituted (Ellis (2—3)) on the mode of insertion of the cilia to ascertain its constancy in the life-history of a species. A culture of a species of *Bacillus* was kept for several months, under different conditions. At frequent intervals cilia preparations were made, and it was found that under all conditions the peritrich ciliation was maintained. The same procedure was followed with a culture of *Pseudomonas*, with the same result, namely, that the polar ciliation characteristic of this genus was also a constant feature. So far as it was possible to conclude from the limited scope of these experiments the mode of ciliation may be used for purposes of classification.

A distinction must also be made between organisms propelled by cilia, and others, like *Beggiatoa alba*, that are motile, but which are apparently without cilia. Even if later researches show that *Beggiatoa* and similar organisms do possess cilia, these are almost certain to be of a different character from the cilia of the bacterial organisms known at present to possess them. Hence it may be possible with further knowledge to make use of this fundamental difference in the mode of ciliation.

Migula in his classification made use of the *number* of cilia. In the author's experience the number of cilia has been found to vary in the same species. The cilia of *Spirillum volutans* were found to vary from one or two up to thirty and even a greater number. The number of cilia seems to have some relationship to the amount of slime formation, and this in its turn depends on the nature of the external cultural conditions, and the other factors which promote or retard growth. Hence the mode of insertion of cilia may be used for classification, but not their number.

(5) *Pleomorphism.*—In Chapter I. it has been shown that some of the sulphur bacteria are pleomorphic; for example, that the organism known as *Lamprocystis* is a form that occurs in the life-history of at least three organisms, namely *Bacterium*

sulfuratum, *Beggiatoa roseo-persicina*, and *Thioporphyra volutans;* and probably of other sulphur bacteria not yet fully investigated. It has also been shown that the zoogloea condition is frequently found in some of the sulphur bacteria; that in some species almost every shape known in the sulphur bacteria may be found; that in some cases the actual transition from one form to another has been directly observed; and that two organisms, apparently specifically distinct, have been observed in organic connection. Hence pleomorphism may be a useful aid in the separation of species, if it can be shown that in certain species its appearance is a regular occurrence.

(6) *Mode of Germination of the Spore.*—The germination of the spore in the genus *Bacillus* is not uniform in all species of that genus. As, however, spore formation, with one or two exceptions, is confined to that genus, this feature cannot be used in the classification of the sulphur bacteria.

(7) *Method of Cell Division.*—With greater knowledge it may be possible to utilize the undoubted differences that exist in the method of division of the cells. In the genus *Bacillus* cell division is preceded by the formation of a plasma-derived wall cutting across the cell. This wall then undergoes longitudinal division, the process resembling in all essentials the method of cell division which is followed in the higher plants. On the other hand, in the genus *Spirillum*, the cell contents withdraw from a certain area, separating the two daughter cells which even at this stage are organically separated; and are kept in place only by a connecting bridge of slime. Complete separation follows the disappearance of the slime (see Ellis (1)). This method occurs where a definite cell membrane is not formed, and it is general among animal cells. With one or two exceptions, *e.g. Beggiatoa mirabilis*, the cells of the sulphur bacteria divide by the second method. This difference in the mode of division could be used with advantage in the classification of the sulphur bacteria were it not that the number in which the first method prevails is so small that its use would not have much value, and that there are too many organisms among the sulphur bacteria of which the exact method of cell division is unknown.

(8) *Intimate Structure of the Cell.*—The structure of the cell is simple and no nucleus is differentiated. In addition, the membrane in the majority of the sulphur bacteria is not well marked (see Chap. X.). So far as is known at present, the presence of sulphur inclusions in the cell does not imply the constant presence of any other solid substance in the cell. The intimate structure of the cell is too uniform in the sulphur bacteria to permit of use being made of it in classification.

(9) *Colour.*—The primary division in all classifications of the sulphur bacteria is based on the presence or absence of colour. The distinction of colour is most useful from the practical point of view, and it is probable also that the differences between the coloured and the uncoloured sulphur bacteria are deep seated. The coloured forms probably utilize the energy of light, as is done by green plants; and if this be the case their metabolism is essentially different from that of the uncoloured forms. To some extent the distinctive features of the coloured and the uncoloured forms are different, but they are not sufficiently well marked to justify their use as definite connotative marks to distinguish one kind from the other.

The terms *Leuco* and *Rhodo* are already in use as prefixes for the colourless and the coloured forms respectively, and it is proposed to retain them, so that the first division in the author's revised system will be

1. *Leuco-thiobacteria.*
2. *Rhodo-thiobacteria.*

A difficulty has arisen in taking this step. The name *Thiospirillum* was originally used by Omelianski for all sulphur spirilla, both coloured and uncoloured. If this primary division on the basis of colour is to be retained, it will be necessary to make a distinction between the coloured and the uncoloured spirilla. To preserve uniformity of nomenclature it is proposed to make the following distinction :—

Thiospirillum—Uncoloured sulphur spirilla.
Rhodothiospirillum—Coloured ,, ,,

Another difficulty arose in the nomenclature of *Thiothrix*. The author has found a coloured *Thiothrix*, which appears to differ in no single particular from the normal uncoloured species, except in colour. Its genetic connection with *Thiothrix* is so patent that it must be retained in that genus in spite of its possession of colour.

(10) *Diversity of Habit.*—Several attempts have been made, notably by Beijerinck and by Orla-Jensen, to frame classifications based on differences in the mode of life of different bacteria. Such attempts would have much to recommend them, were it not that the majority of bacterial organisms could be included not in one but in several groups. It has already been pointed out that many parasites, like *Bacillus cholerœ* for instance, lead a saprophytic life when living cells are not available. Many other instances may be cited in support, and it may be stated of bacteria in general that the more that is known of their metabolism the greater the conviction of the diversity of their habits. All physiological systems fail to satisfy the first requisite of a logical classification, namely, that it should not be possible to place an organism in more than one group. The sulphur bacteria are not cultivable on ordinary media, so it is not possible to use such cultural characteristics as gelatine liquefaction, or sugar reactions, for the diagnosis of species. The reaction of the sulphur bacteria to the Gram stain has not yet been investigated, so that its use is problematical.

Summary.

The foregoing remarks may now be summarized as follows :

(1) *Spatial Division of the Cells.*—Of limited application.

(2) *Motility.*—A positive result is valuable, but a negative one is not. Absence of movement may be due to an abnormal development of slime, with a consequent restriction of cilium development. Caution must therefore be exercised. The sulphur bacteria may be divided into three groups on the basis of motility : (i) Motile with cilia ; (ii) Motile without cilia ; (iii) Motility unknown.

(3) *Size of Cells.*—Of limited application, on account of the variations both in length and in thickness. Certain ranges of dimensions may be given, if they are not regarded as having an absolute value.

(4) *Ciliation.*—The mode of distribution of cilia is constant. Bacteria may be divided into three classes on this basis: (i) With peritrich ciliation; (ii) with polar ciliation; (iii) with no cilia. The number of cilia is not constant, except where an organism has not more than one cilium.

(5) *Pleomorphism* constitutes a factor which may be used in the grouping of the sulphur bacteria. Our knowledge of this phenomenon in the sulphur bacteria is at present too scanty to make much use of it.

(6) *Mode of Germination.*—Not applicable.

(7) *Method of Cell Division.*—Two methods are known: (i) with formation of transverse wall in the plasma; (ii) without such a formation. The value of this difference is considerably lessened because practically the whole of the sulphur bacteria belong to the second class.

(8) *Intimate Structure of the Cell.*—The differences are not sufficiently marked to give them value for grouping purposes.

(9) *Colour.*—The presence or absence of colour is the most important distinction that is found in the sulphur bacteria.

(10) *Diversity of Habit.*—As many, perhaps all, bacteria do not follow only one mode of life, a grouping based on the mode of life of the sulphur bacteria can have very little value for the major partitions, although there is a possibility of its use in the minor divisions.

There are thus many difficulties to encounter in the classification of the sulphur bacteria. The confused state of the classifications of the present day arises from the bestowing of names on what appear to be new species, after only an inadequate investigation. Genera have been founded on characters whose constancy is open to question; and very little pains have been taken to ascertain whether the life-history of the organism under investigation is limited to the one phase that is presented to the observer.

Probably more classifications of bacteria have been issued

within the last thirty years than of any other class of plants; and yet there has been practically no critical examination of the soundness of the characters that have been used to effect the division of these organisms. The classification sponsored by the American Society of Bacteriologists is no exception, at any rate in that part of it which relates to the sulphur bacteria. It is proposed in the present work to attempt a remedy in the limited field of the sulphur bacteria, and to modify Buchanan's classification after elimination from it of what is regarded as of dubious value.

Review of Previous Classifications.

Winogradsky's Classification of the Sulphur Bacteria.

The predecessors of this grouping may be left out of account, as they were based on very scanty and inaccurate knowledge.

Winogradsky, 1888, distinguished fourteen genera, including the genera of previous investigators:—

I. Colourless Bacteria.

(1) *Beggiatoa.*—Threads equally thick, freely motile, and not forming gonidia.

(2) *Thiothrix.*—Threads unequally thick, attached, and forming motile gonidia.

II. Coloured Bacteria.

(A) *Cells united in families.**

a. Division in three directions of space.

(3) *Thiocystis.*—Single or several families of small cells, surrounded by a slime cyst, and capable of movement.

(4) *Thiocapsa.*—Several families of round cells, loosely embedded in a common envelope of slime. Not motile.

(5) *Thiosarcina.*—Families in packets.

b. Division at first in three, later in two directions of space.

* The term *family* is used here in the sense of a *cluster* or an *aggregate* of similar cells.

(6) *Lamprocystis.*—Families at first forming a solid mass, later forming a reticulated hollow sphere.

c. Division in two directions of space.

(7) *Thiopedia.*—Families in plates, cells arranged symmetrically in fours, and capable of motion.

d. Division of cells in one direction of space.

(8) *Amœbobacter.*—Families amœboid and motile. Cells connected by plasma threads.

(9) *Thiothece.*—Families with thick slime cysts. Cells motile and loosely enclosed in a common envelope of slime.

(10) *Thiodictyon.*—Families made up of rods arranged netwise.

(11) *Thiopolycoccus.*—Families compact. Cells non-motile, small and closely pressed together.

(B) *Cells free, and capable of movement.*

(12) *Chromatium.*—Cells cylindrical-elliptical.

(13) *Rhabdochromatium.*—Cells rod- or spindle-shaped.

(14) *Thiospirillum.*—Cells spirally twisted.

Remarks on Winogradsky's Classification.

The attributes selected for the grouping are simple, and for the most part easily ascertainable. The most serious fault of this system lies in the inconstancy of the characters used in the grouping, and this applies in particular to the partition of these organisms according to the number of dimensions in space in which they divide. Motility or its absence is also an uncertain factor.

The following features in the species are constant :—

(*a*) The division into coloured and uncoloured forms.

(*b*) The division into free cells and aggregates of cells (families).

(*c*) The shape of the cell (rod, globular, etc.)

As Winogradsky discounted the facts of pleomorphism, and in consequence magnified the importance of variants into species and even into genera, the value of his system is

considerably impaired. Of the fourteen genera the generic names *Lamprocystis*, *Thiosphærion*, and *Thiopolycoccus* have been discontinued for reasons given later. The remaining eleven genera are retained with modifications. Since the publication of Winogradsky's system a comparatively large number of new genera have been added.

Both the defects of Winogradsky's scheme and the discovery of new genera which do not readily fit into his scheme, make it necessary to modify the bases of grouping used by him.

This classification is, however, one of great importance since it has formed the pivot about which have turned the majority of subsequent systems. The genera established by Winogradsky have been incorporated into the general schemes of classifications of bacteria. They appear with a few modifications in the elaborate classifications of De Toni and Trevisan (1889), Hansgirg (1888), Cornil-Babes (1890), Migula (1890 and 1894), Ludwig (1892), Sternberg (1892), Fischer (1895), Lehmann and Neumann (1896), Chester (1897), Kendall (1902), and Molisch (1907). During the period 1888-1907 there were no investigations to determine the validity of the features selected by Winogradsky for the grouping of the sulphur bacteria. Their diagnostic value had not been challenged, and in all, the facts of pleomorphism were either unknown, or if known, disregarded. The most noteworthy contribution to our knowledge of the sulphur bacteria during this period was Molisch's *Die Purpurbakterien*, and in the system established by him notable changes were introduced.

During the period 1888-1907 a new genus of colourless sulphur bacteria was discovered by Hinze (1903), and named *Thiophysa volutans*. No consideration, however, was given to this organism by Molisch because this writer dealt only with the coloured sulphur bacteria.

Molisch's Classification of the Sulphur Bacteria.

Order *Rhodobacteria*.

Bacteria coloured red, rose, violet, or carmine, by the presence of bacteriopurpurin.

1. Cells contain free sulphur.

Family (1) *Thiorhodaceæ.*

(*a*) Cells united into families.

Sub-family *a. Thiocapsaceæ.*—Division of cells in three directions of space.

Sub-family *b. Lamprocystaceæ.*—Division of cells first in three, then in two directions of space.

Sub-family *c. Thiopediaceæ.*—Division of cells in two directions of space.

Sub-family *d. Amœbobacteriaceæ.*—Division of cells in one direction of space.

(*b*) Cells always swarming.

Sub-family *e. Chromatiaceæ.*—Not capsulated.

Sub-family *f. Rhodocapsaceæ.*—Capsulated.

2. Cells do not contain free sulphur.

Family (2) *Athiorhodaceæ.*

(*a*) Cells united into families.

Genus *a. Rhodocystis.*—Cells rod-shaped, many being embedded together in a common capsule.

Genus *b. Rhodonostoc.*—Cells spherical or short rods in chains, each chain enclosed in a capsule.

(*b*) Cells free.

Genus *a. Rhodococcus.*—Cells spherical, non-motile.

Genus *b. Rhodobacterium.*—Cells straight rods and non-motile.

Genus *c. Rhodobacillus.*—Cells, motile rods.

Genus *d. Rhodovibrio.*—Cells, short, bean, or comma shaped; monotrichous, actively motile.

Genus *e. Rhodospirillum.*—Cells, spiral; actively motile; polar ciliation.

Remarks on Molisch's Classification.

Molisch did not investigate the *colourless* sulphur bacteria, and in the present work we are not dealing specifically with the sulphurless purple bacteria. His classification has

therefore only a partial application. He divides the coloured sulphur bacteria into six groups, the first four of which are divided according to the number of directions in space in which the cell divides, and the last two are separated from the rest by being free instead of forming families. This classification, in one respect, is not an advance on that of Winogradsky, for in it also the number of dimensions in space in which an organism divides is made the basis of the primary grouping.

Molisch reduced Winogradsky's fourteen groups to six, as he omitted the following: *Thiocystis*, *Thiosarcina*, *Thiothece*, *Thiodictyon*, *Thiopolycoccus*, *Rhabdochromatium*, and *Thiospirillum*. No reasons are given except that the classification is made "auf Grund meiner Erfahrung." As Molisch's researches did not deal, except incidentally, with the grouping of the sulphur bacteria, his classification of these forms is necessarily incomplete. He, however, added two genera to the sulphur bacteria, namely *Rhodocapsa* and *Rhodothece*. Both are sharply marked off from all the genera hitherto included in the sulphur bacteria. In his own classification they are included presumably in Rhodocapsaceæ, although he does not specify the genera of this sub-family in his scheduled classification.

A notable departure in the method of classification of bacteria was made by Orla Jensen (1909). In the interval the list of sulphur bacteria had been supplemented by the discovery of the genus *Thioploca* by Lauterborn (1907). Orla Jensen introduced entirely new factors in his classification. Whilst using morphological characters for the main groupings, the families and genera are separated on the basis of physiological differences.

Orla Jensen's Classification.

Two Orders are distinguished.

1. *Cephalotrichinæ*.—Cells spherical, rod-shaped, or spiral. Endospores formed only in a few sulphur-free spirilla. When motile, mono- or lopho-trichous. Typically water bacteria. Energy almost exclusively by oxidation processes.

2. *Peritrichinæ.*—Cells spherical, or rod-shaped, never spiral. Either peritrichous or non-motile. Not typically water bacteria. Energy not by oxidation processes.

Seven families are included in the first order :—

Oxydobacteriaceæ.
Luminibacteriaceæ.
Reducibacteriaceæ.
* *Thiobacteriaceæ.*—Containing sulphur but no bacteriopurpurin.
* *Rhodobacteriaceæ.*—Containing sulphur and bacteriopurpurin.
Actinomyces.
* *Trichobacteriaceæ.*—Water forms. Absence of mycelium. Filamentous.

Four families are included in the second order :—

Acidobacteriaceæ.
Alkalibacteriaceæ.
Butyribacteriaceæ.
Putribacteriaceæ.

All the sulphur bacteria then known were placed in one or other of the three families marked with an asterisk.

Family *Thiobacteriaceæ :*

1. Cells not spiral.
   A. Cells rod-shaped or not spherical.
      * *Sulphomonas.*—Autotrophic, rod shaped.
      Thiomonas.—Heterotrophic, oval.
   B. Cells spherical.
      Thiococcus.—Cells spherical.
2. Cells spiral.
      Thiospirillum.

Family *Rhodobacteriaceæ :*

Rhodomonas (*Chromatium*).
Rhabdomonas (*Rhabdochromatium*).

* *Sulphomonas* is not rightly included in *Thiobacteriaceæ* as the cells do not store sulphur.

Rhododictyon (*Thiodictyon*).
Amœbomonas (*Amœbobacter*).
Rhodothece (*Thiothece*).
Rhodopolycoccus (*Thiopolycoccus*).
Rhodococcus (*Thiopedia*).
Lamprocystis.
Rhodocystis (*Thiocystis*).
Rhodocapsa (*Thiocapsa*).
Rhodosarcina (*Thiosarcina*).
Rhodospirillum (*Thiospirillum*).

Family *Trichobacteriaceæ*:

1. Not containing sulphur granules.
2. Containing sulphur granules.
   a. *Beggiatoa*. Threads not attached. Motile.
   b. *Thiothrix*. Threads attached.

Remarks on Orla Jensen's Classification.

All the fourteen genera introduced by Winogradsky were accepted, but their names were changed. In addition four new genera were added. The first of these calls for particular attention, because it is a genus composed of cells in which sulphur is not stored. This is *Sulphomonas*, the name in this system for the thionic acid bacteria, which by this time had been discovered by Nathansohn. *Thiophysa volutans* appears under the name *Thiomonas*. The third and fourth genera, namely *Thiococcus* and *Thiospirillum*, were added as logical sequences to the insertion of rod-shaped genera in the family *Thiobacteriaceæ*. The term *Thiospirillum* was not well chosen, as it had already been used by Winogradsky with a different meaning.

It has already been affirmed that all classifications with physiological attributes as the main basis of the grouping cannot be regarded as satisfactory, because bacteria are not confined to only one mode of life.

Buchanan's Classification (1917).

He distinguishes six orders of the class *Schizomycetes*.

Order 1.—*Eubacteriales.*
Order 2.—*Chlamydobacteriales.*
Order 3.—*Actinomycetales.*
Order 4.—*Thiobacteriales.*
Order 5.—*Myxobacteriales.*
Order 6.—*Spirochætales.*

He gives the following key to the families of the order *Thiobacteriales*, which includes all the sulphur bacteria.

Family (1) *Achromatiaceæ*.—With sulphur, but no purpurin.

Unicellular: not motile, and not filamentous.

Genus 1. *Achromatium*.—Cells ellipsoidal, containing calcium oxalate, and perhaps sulphur.

Genus 2. *Thiophysa*.—Spherical cells, with sulphur granules in a central vacuole.

Genus 3. *Hillhousia*.—Cells longer, and very large (42—86μ), with peritrichous cilia.

Family (2) *Beggiatoaceæ*.—Like family (1), but filamentous.

Genus 1. *Thiothrix*.—Non-motile filaments, thread unequally thick; attached.

Genus 2. *Beggiatoa*.—Filaments, motile, not attached, thread cylindrical; filaments not in bundles, nor surrounded by a gelatinous sheath.

Genus 3. *Thioploca*.—As Genus 2, but filaments in bundles surrounded by a gelatinous sheath.

Family (3) *Rhodobacteriaceæ*.—Containing bacteriopurpurin, and with or without sulphur granules. These he divides into two sub-families:—

Chromatioideæ.—Containing sulphur granules.
Rhodobacterioideæ.—Without sulphur granules.

Chromatioideæ.—Divided into five tribes as follows:—

Thiocapseæ.—Cells in families: division in three directions of space.

Lamprocysteæ.—Cells in families : division first in three, then in two directions of space.

Thiopedieæ.—Cells in families : division in two planes, forming plates of cells.

Amœbobacteriaceæ.—Cells in families : division in one plane.

Chromatieæ.—Cells free, capable of swarming.

(It is not necessary to consider the *Rhodobacterioideæ* in this work, as they are not sulphur bacteria.)

Key to the *Thiocapseæ :*

Genus 1. *Thiocystis.*—Motile : families small, compact, enclosed singly or several, in a cyst.

Genus 2. *Thiosphæra.*—Motile : cells large (7—8μ), loosely bound by gelatine into loose families.

Genus 3. *Thiosphærion.*—Motile : cells small, united to form solid, spherical families.

Genus 4. *Thiocapsa.*—Non-motile : cells spread out in flat families, loosely enveloped in a common gelatine.

Genus 5. *Thiosarcina.*—Non-motile : cells in regular packets.

Key to the *Lamprocysteæ :*

One genus *Lamprocystis.* As for family.

Key to the *Thiopedieæ :*

Genus 1. *Lampropedia.*—Cells arranged regularly in fours.

Genus 2. *Thioderma.*—Cells in a film, or membrane, not regularly disposed in tetrads.

Key to the *Amœbobacterieæ :*

Genus 1. *Amœbobacter.*—Cells connected by plasma threads : families amœboid, and motile.

Genus 2. *Thiodictyon.*—Cells arranged in a net by their ends.

Genus 3. *Thiothece.*—Cells not arranged in a net, and loosely aggregated in gelatine : motile.

Genus 4. *Thiopolycoccus.*—Same as the preceding, only cells are not motile, and closely appressed into a colony.

Key to the *Chromatieæ:*

Genus 1. *Chromatium.*—Cells motile, with polar cilia and cylindrical.

Genus 2. *Rhabdomonas.*—Same as preceding, only cells have a tendency to a spindle shape.

Genus 3. *Thiospirillum.*—Same as above, only cells are spiral.

Genus 4. *Rhodocapsa.*—Cells spherical, or nearly so, and non-motile, not capsulated.

Genus 5. *Rhodothece.*—Same as preceding, only cells capsulated, and in pairs.

Remarks on Buchanan's Scheme of Classification.

In the above scheme only that part which deals with the sulphur bacteria is here considered. All the members of this group are included in the order *Thiobacteriales*, which is one of the six into which the *Schizomycetes* are divided. There are twenty-two genera in this order, but no new species of sulphur bacteria.

The different genera of the sulphur bacteria are all included in the one order. It is evident that the classification of the sulphur bacteria has been drawn up without specific investigation of these organisms, and without discrimination between the values of various attributes. The characters chosen for effecting the grouping are merely mixtures of the characters used in previous classifications. The result is naturally the perpetuation of all the errors that are found in the previous schemes. Whilst Buchanan's scheme appears to be more complete than older classifications, it is not, so far as the sulphur bacteria are concerned, and in essentials is no advance on Winogradsky's plan.

In the scheme drawn up under the ægis of the American Society of Bacteriologists, the grouping of the sulphur bacteria follows very closely the plan laid down by Buchanan. New generic and specific names appear, but as these did not appear as the result of independent investigations, the scheme is cumbersome without compensating advantages.

A committee of the Society of American Bacteriologists (Bergey, Breed, Hammer, Harrison, and Hunton) prepared a *Manual of Determinative Bacteriology* in 1923, and in the classification of the sulphur bacteria they have adopted Buchanan's scheme without change. The same applies to Pribram's classification which appeared in 1929.

Ellis's Classification of the Sulphur Bacteria.

Buchanan's classification has the merit that it takes cognizance of all the sulphur bacteria that have been discovered in recent years. The present writer's scheme has been drawn up after an inspection of the stability of the attributes used in the classification of bacteria in general, and of the sulphur bacteria in particular. Also the life-histories of these bacteria have been studied, and observations have been made on the majority of the organisms included in this group. In this way the defects of the earlier systems have been made manifest, and so far as has been possible, they have been avoided. As a quarter of a century has elapsed since a scheme of classification of the sulphur bacteria has been drawn up after a special investigation of the group, there is much to rectify. The acceptance of pleomorphism is a fact of great importance in framing a scheme of division. The instability of dimensional division in space is also a fact that strikes at the roots of former classifications, because of the importance attached to it. The genus *Lankesteron* has been created because it was felt that the organisms examined respectively by Lankester, Warming and Zopf were different species of a highly pleomorphic genus of the sulphur bacteria. Within the last twenty-five years, since the last classification was made after a detailed study of the group, several additions have been made to the group, and the author has added still another genus, namely, *Thioporphyra*. In addition a slight change has been made for the ake of preserving uniformity of nomenclature. The Spirilla genera of the sulphur bacteria have been divided into two, namely, *Thiospirillum* (colourless), and Rhodo-thiospirillum (coloured). The term Thio-pseudomonas was also introduced

for purposes of uniformity. It has been found necessary to discard some hitherto familiar names. The best known of these is *Lamprocystis*. It has been shown already (p. 18) that the organisms known by this name are forms of growth of one or other of several organisms, and not independent species. It is considered that the attributes by which this genus is known, namely, that division is at first in "three directions of space" and subsequently in "two directions of space," is a fugitive character, and due in all probability to some change in the constitution of the slime which envelops the cells. The validity of this alleged distinctive feature of Lamprocystis has never been confirmed by any subsequent writer.

The following genera are discarded for reasons given below:—*Thiosphæra*, *Thiosphærion*, *Thiothece*, *Thiopolycoccus*, and *Hillhousia*. Of these the first four are discarded because it is considered that they represent merely phases in the life-histories of various pleomorphic bacteria. Zoogloea structures, when they occur in organisms other than the sulphur bacteria, have not been regarded as other than forms of growth, not as separate organisms. There is still some doubt whether the genus *Hillhousia* is specifically distinct from *Achromatium*. The balance of evidence supports the view that they are identical.

Ellis's Classification.

The sulphur bacteria are divided into two groups:—

A. *Leuco-Thiobacteria.*—Colourless sulphur bacteria.

B. *Rhodo-Thiobacteria.*—Coloured sulphur bacteria.

A. *Leuco-Thiobacteria.*—Four families.

Family 1. *Beggiatoaceæ.*—Normally straight filaments, but may show spiral or globular forms; normally motile; occasionally enters zoogloea condition.

Genus 1. *Beggiatoa.*—Normally straight, motile, filaments.

Genus 2. *Thiothrix.*—Filaments attached, and often enclosed in sheath of hardened slime.

Genus 3. *Thioploca.*—Aggregates of threads in hardened slime.

Family 2. *Achromatiaceæ.*—Free, motile, cells normally spherical or spheroidal in form: reproduction by fission and by zoospores.

Genus 1. *Achromatium.*—Cylindrical, ellipsoidal, cells; reproduction by fission and by zoospores; movement chiefly of translation and slow.

Genus 2. *Thiophysa.* Cells globular, *or ovoid*, uni- and diplo-cocci common; movements by translation and rotation; reproduction by fission.

Genus 3. *Thiosphærella.*—Ellipsoidal cells; thick membrane surrounded by slime; movement slow; reproduction by fission.

Genus 4. *Thiovulum.*—Oblate spheroid or this figure with one end slightly elongated; reproduction by fission and division at right angles to long axis.

Family 3. *Thiospirillaceæ.*—Free, spiral, cells; polar cilia.

Genus 1. *Thiospirillum.*—Free, motile, spiral, cells; division by fission.

Family 4. *Thiobacillaceæ.*—Short rods; division by fission.

Genus 1. *Thiobacillus.*—Peritrich ciliation.

Genus 2. *Thiopseudomonas.*—Polar ciliation.

B. *Rhodo-Thiobacteria.*—Seven families.

Family 1. *Lankesteraceæ.*—Normally filamentous; highly pleomorphic, with rod, spiral, and coccal, forms; occasionally forms zoogloeæ.

Genus 1. *Lankesteron.*—As for family.

Family 2. *Chromataceæ.*—Spherical, or ellipsoidal, free cells; one polar (probably compound) cilium.

Genus 1. *Chromatium.*—Rose-pink colour; uni-cocci; reproduction by fission, and probably also by sexual cells.

Genus 2. *Thioporphyra.*—Purple colour; uni-, diplo-, and tri-cocci; reproduction by fission, by budding, and probably by endospores.

Family 3. *Rhodothiospirillaceæ.*—Free, spiral, cells; polar ciliation.

Genus 1. *Rhodothiospirillum*, as for family.

Family 4. *Rhodocapsaceæ.*—Free cells each surrounded by slime; motile.

Genus 1. *Rhodocapsa.*—Uni- or diplo-cocci, or short chains.

Genus 2. *Rhodothece.*—Rod cells; contain ærosomes.

Genus 3. *Rhodosarcina.*—Cocci arranged in packet form.

Family 5. *Thiocapsaceæ.*—Globular cells, forming clusters enclosed in slime.

Genus 1. *Thiocapsa.*—Cells non-motile on escape from slime.

Genus 2. *Thiocystis.*—Cells motile on escape from slime.

Genus 3. *Thiosphærion.*—Violet colour; slime colonies of globular form; cells motile on escape from slime.

Family 6. *Amœbobacteriaceæ.*—Rods or spherical cells enclosed in slime; cells connected by plasma threads.

Genus 1. *Amœbobacter.*—Sphæroidal cells in slime which move in unison when they separate or close in.

Genus 2. *Thiodictyon.*—Rod cells united like a Hydrodictyon net.

Family 7. *Thiopediaceæ.*—Globular cells in slime arranged in regular formation.

Genus 1. *Thiopedia.*—As for family.

CHAPTER VI.

LEUCO-THIOBACTERIA (COLOURLESS SULPHUR BACTERIA).

Family 1. *Beggiatoaceæ*. Genus 1. *Beggiatoa;* Genus 2. *Thiothrix;* Genus 3. *Thioploca.*

The *Leuco-Thiobacteria.*—This group includes all the colourless sulphur bacteria.

Family 1. *Beggiatoaceæ.*

Straight filaments sometimes spiral or rounded. The filaments are unicellular, and either homogeneous or divided into sections by the formation of thin transverse bands of hyaline slime at more or less regular intervals. Slime is formed outside the membrane. In *Beggiatoa* slime formation is scanty; in *Thiothrix* the slime hardens and forms a cylindrical sheath with transverse bands; in *Thioploca* numerous filaments are enclosed in a well-developed mass of slime.

The filaments are motile, the degree of motility being determined chiefly by the amount of slime formation.

Reproduction is by fission, and in one genus (*Beggiatoa*) also probably by asexual spores.

Genera—

1. *Beggiatoa.*
2. *Thiothrix.*
3. *Thioploca.*

Genus 1. *Beggiatoa* (Trevisan), 1842.

Colourless threads, non-septate, and typically motile. One species (*B. alba*) shows high degree of pleomorphism. The varieties of form include filaments, large and small cocci, short rods, and ovoid structures. These may be motile or

non-motile, and may be either free or enclosed in slime (zoogloea condition).

The developmental life-history and methods of reproduction are fully known only in *B. alba*. In this species reproduction is by fission, and probably also by formation of endospores. Raw cultures in nature form a white slimy or felted cover on the surface of various objects undergoing decomposition; or if the organism is growing in sewage-laden water, on the bed of the stream.

Beggiatoa alba (Vaucher), Trevisan (1), 1842.

Synonyms.—*Oscillaria alba* (Vaucher); *Hygrocrocis Vandellii* (Meneghini); *Beggiatoa punctata* (Trevisan); *Beggiatoa media*, and *Beggiatoa minima* (Winogradsky).

Literature.—Vaucher, 1803; Meneghini (1833-36); Trevisan (1842); Cohn (10), 1875; Warming, 1875; Zopf (1), 1882; Winogradsky (2), 1888; Bütschli (1), 1890; Fischer (1), 1891; (3), 1897; Mitrophanow, 1893; Corsini, 1905; Keil, 1912; Ellis (1924, 1927); Bergey, 1924; Bavendamm, 1924.

Description.—In mass cultures *Beggiatoa alba* typically forms a dark grey, loosely textured, felted shroud, covering the surface of its host: or if the water is sewage-contaminated a covering on the bed of the stagnant or slow-moving water. *Beggiatoa* is typically made up of long threads of cylindrical form with rounded ends, and unless very long these are of uniform thickness, 2—6μ. The movement is normally slow and without undulation. Lengths of even a centimetre and more have been observed.* Long threads do not move forward but exhibit a slow swinging jerky movement at the ends, resembling that of *Oscillatoria*. The average length is 50—100μ. The cells contain, under favourable conditions, an abundance of sulphur globules.

Motile, colourless threads, with oil-like contents, in a water containing organic matter in solution, may with certainty be referred to Beggiatoa alba.

* A filament of this length is on the same scale as an inch-thick rope about 140 yards long; a remarkable instance of cohesion in a substance of such soft matter.

Sheath Formation.

The existence of a sheath-covering in *Beggiatoa* was recorded as early as 1884 by Winter, in Rabenhorst's *Kryptogamen Flora;* and in more recent work it is mentioned by Selk, 1907, and by Keil, 1912. Further, Koppe, 1923, has recently shown that *Beggiatoa arachnoidea* occasionally becomes attached to objects in the water by the development of cushions of slime at one end of the thread. The occurrence of slime formation, however, as a normal feature in the life-history of *Beggiatoa alba* has not hitherto been recognized.

Sheath formation is characteristic of all conditions of the *Beggiatoa* cell, but in motile threads its amount is too slight to be perceptible without special treatment. A similar formation is found in the iron bacteria, *e.g. Crenothrix polyspora*, but whereas in these the sheath formation is a normal occurrence of healthy cells, in *Beggiatoa alba*, as in yeast, *active* slime formation is abnormal. The autolysis of the cells is always accompanied by excessive slime formation (see below). The thread appears to be divided transversely by walls such as are normally formed in the division of cells of the higher plants. In this case, however, they are merely transverse bands of hardened slime. Their mode of formation is described below.

Growth.

Under favourable conditions a thread grows to double its length in twenty-four hours, and during this time undergoes one division. According to Winogradsky, this rate of growth continues as long as the thread receives an adequate supply of sulphuretted hydrogen. The amount of this substance daily absorbed by a normal thread is substantially greater than the amount of protoplast in that thread. Growth is intercalary.

Autolysis.*

Mass cultures of *Beggiatoa alba* in nature usually disappear completely with the advent of unfavourable circumstances.

* *Autolysis* refers to a process initiated inside the cell which results in the disappearance of the organism by self-liquefaction.

The filaments frequently suffer dissolution with dramatic suddenness, for the whole procedure which is detailed below may occupy not more than a few minutes. The procedure in autolysis follows one of two courses.

First Method.—After coming to rest the filament breaks into fragments, as shown in Fig. 6*c*. The sheath at this stage can be made visible by treatment with iodine. Between the short lengths into which the thread has divided (Fig. 6*d*) transverse sheaths of slime are formed. Then, beginning with one of the cells at or near the middle, each swells in turn (Fig. 6*e*). The order of swelling seems to be strictly maintained. By the time the fourth or fifth has begun to swell, the starting cell has completely disappeared. The process continues until the last cell has dissolved.

Second Method.—When autolysis takes place in a filament in which a well-developed and hardened sheath has already been formed, a change in some of the details of the process is observed. As before, the thread breaks up into a number of short lengths, but the fragmentation is accomplished inside the hardened sheath (Fig. 6*h*) ; and it is within the sheath that the dissolution of the cells is effected. In Fig. 6*h* is shown an example in which all except three cells have disappeared. In this figure a septum of slime is also shown.

Cause of Autolysis.—Winogradsky (2) states that autolysis takes place as a result of the deprivation of sulphuretted hydrogen, but he did not advance any proof in support of this statement. Actually, it takes place when inimical conditions of growth reach a certain stage of intensity, and this may result not only from the dearth of sulphuretted hydrogen but also from an insufficiency or an excess of oxygen, or from other causes. When the conditions of growth are not satisfied the filaments disappear, so that in most cases not one but several factors are concerned. It has been suggested that the process is furthered by the secretion of a specific ferment, but no proof of the existence of such a ferment has been advanced. It is very unlikely that a process which is accompanied by a series of remarkable morphological changes originates or is furthered by a ferment. It must be borne in mind that the

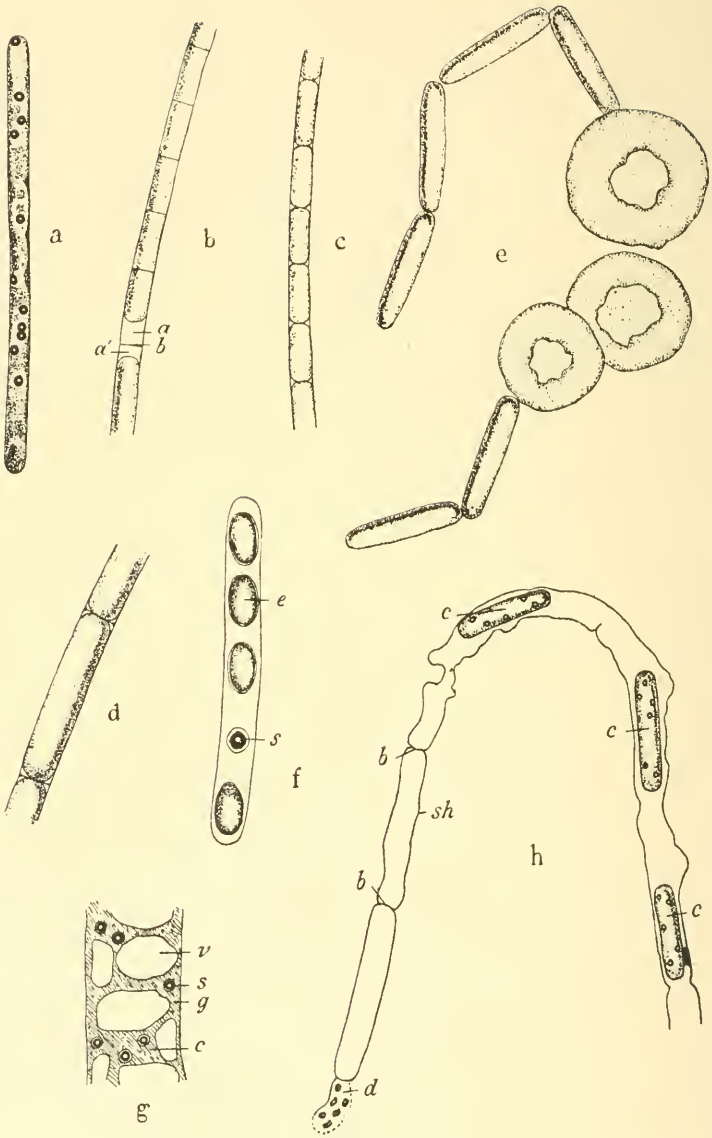


FIG. 6.

protoplast segments into a number of equal or approximately equal lengths, and that the morphological changes outlined above take place in a regular sequence. Autolysis must be regarded as a *vital* phenomenon, as much so as the capacity of a plant to respond to such outside influences as light, gravity, etc. In the course of this response ferments may or

FIG. 6.—*Beggiatoa alba* (Vaucher). Trevisan (1), 1842.

a.—Photomicrograph (slightly touched up) of a short filament containing a few granules of sulphur. × 1200.

b.—Fragment of thread undergoing autolysis. Transverse walls are clearly visible. At one point the protoplast has receded from both sides of the transverse band of sheath (at *b*) leaving a clear space. This manner of separation is characteristic of the process. × 1500.

c.—Shows the first stage in autolysis. The thread has broken up into fragments of approximately equal lengths. × 1500.

d.—Second stage in autolysis. Transverse bands of hardened slime are seen between the segments. × 2000.

e.—Third and final stage in autolysis. Nine segments are shown, of which three are in process of swelling, preparatory to dissolution. × 1500. (For further explanation see text.)

f.—Short filament with four endospores. × 1500.

e—Endospore.

s—Sulphur globule, somewhat contracted, lying in its vacuole.

g.—Semi-diagrammatic representation of a portion of thread to show alveolar arrangement of the plasma. × 4500.

v—Vacuole.

s—Sulphur globules.

g—Minute granules in plasma.

h.—Filament in last stage of autolysis. Whilst the sheath has remained comparatively unchanged, its contents have almost disappeared. × 1500. (For further information see text.)

c—Remains of the disappearing segments.

sh—Sheath.

b—Transverse band of sheath-material.

d—Debris left after disappearance of another portion of the filament.

may not be developed, but if formed they are to be regarded as concurrent, not as causative agents in the production of the phenomenon, and their presence as an incidental, not as a necessary feature.

The mechanism of the process is a physical one. All living cells contain osmotically active substances, and normally, equilibrium is maintained because the outward pressure is

balanced by the resistance of the somewhat hardened and chemically changed outermost layers of the protoplast. The balance is evidently a very delicate one, and one which is easily destroyed by a further change in the constitution of the resisting outer layers when these are transformed into slime. This causes the outwardly directed pressure to overcome the resistance of the outer layers, with the results described above. It is as though the leather casing of an inflated football were gradually changed into a softer material. Expansion would occur and ultimately there would be a complete disappearance of the ball. A hypothetical ferment may be predicated to bring about the transformation of the outermost layers, but there is no necessity to assume its existence to *explain* what in the last resource is a vital phenomenon, which does not necessarily demand the existence of a ferment for its manifestation.

Methods of Reproduction.

(A) *Fission.*—The normal method of reproduction is by fission. At first the two daughter cells are attached by a band of slime continuous with the surface slime. This is made clearer on staining the slime with iodine or carbol-fuchsin. Later the daughter cells gradually draw apart, but the continuity of the slime is maintained until the cells are about their own length apart.

Zopf (2) states that the separation is facilitated in stationary threads by the swinging movements of the free end, and Winogradsky (2) affirms that a break usually occurs at a place on the thread where a large vacuole is found.

(B) *Endospores.*—Structures similar to endospores were observed on one occasion inside the threads of a *Beggiatoa* growing in a pool on the rocks near the Millport Marine Biological Station, Scotland.

The following reasons suggest that these structures were spores :—

(*a*) Constancy of size and shape (4μ long and 3·5μ broad).

(*b*) Difficulty of staining.

(*c*) The approximation of the size to that of the normal bacterial endospore in the genus bacillus.

Their germination has not been observed.

Sexual reproduction apparently does not occur.

Species of the Genus *Beggiatoa*.

When many individuals of *Beggiatoa* are observed in the same microscope-field, threads varying in thickness from 1μ and less to approximately 3μ are found, and a similar variation is to be found in the lengths of the cells in the threads.

Winogradsky (2) has arbitrarily fixed on certain ranges of size in such a mixture and given a specific name to each selected range. He distinguishes different species in accordance with the dimensions given in the following table:—

	Thickness in μ.	Length of Cells in μ.
Beggiatoa alba	4—5·5	2·9—5·8
,, media	1·0—2·5	4·0—8·5
,, minima*	Up to 1μ	—

A criticism of this procedure is given on page 72.

Pleomorphism of *Beggiatoa alba*.

Zopf (1) has found this organism to be highly pleomorphic: it can exist in a motile free state, or in a non-motile attached form. The long threads show a differentiation of base and apex.

The filament breaks up under certain conditions and forms a mass of cocci.† In Fig. 7*a* is shown a filament which has

* The name *Beggiatoa minima* has been applied to two different organisms, this name being given by Winogradsky to one organism, and by Warming to another. That described by Warming has since been taken away from the Bacteria by Lauterborn, and placed in another group under the name *Spirophis minima*.

† Zopf uses the term *coccus*, not to designate an individual belonging to the group *Coccaceæ*, but to indicate a small rounded mass of plasma. In the accepted use of this term nowadays, a coccus is a spherical mass of plasma surrounded by a definite cell membrane.

already assumed an irregular form preparatory to breaking up, and which is already showing at its basal end small independent masses of plasma, each containing a few grains

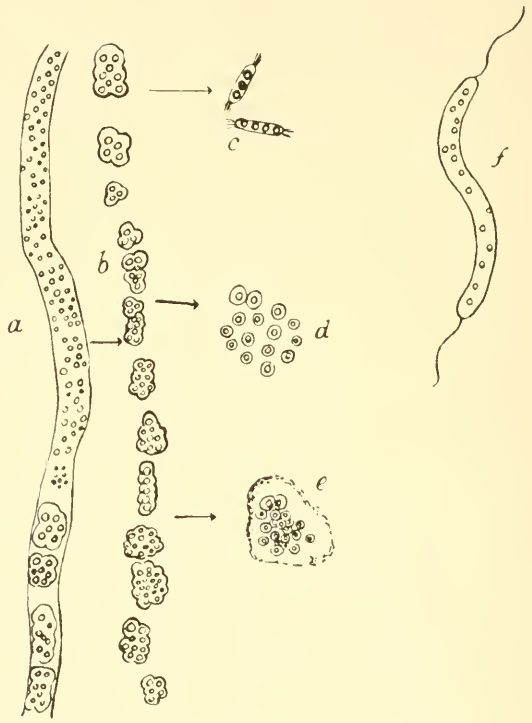


FIG. 7.—Pleomorphism of *Beggiatoa alba*.

At *a* is shown a filament breaking up into separate clumps of plasma, each including a number of sulphur granules. In the lower part of the figure, four of these clumps are shown.

In *b* the formation of these clumps is shown completed, although the alignment of the filament is still maintained.

In *c*, *d*, *e* are shown the different forms into which the members of the clumps develop, namely, free cocci, short rods, or zoogloea masses. Either cocci or short rods may be found in the zoogloea masses.

At *f* is shown a rigid spiral cell formed by separation from the filament.

of sulphur. In the next state (Fig. 7*b*) the filament has given place to a number of separate clumps of plasma. Next, the clumps break up into *micrococci* which may become motile in

that form, or after they have developed further to the rod form (Fig. 7*c*). Or the cocci may multiply and enter into the zoogloea condition (Fig. 7*e*). The last-named condition may be brought about by inoculating material rich in *Beggiatoa* into a fluid made up of a mass of *Spirogyra* and *Cladophora* in a partial state of putrefaction in water. Also Zopf observed a fragment of a filament being cut off from the end, when it assumed a twisted form and swam away (Fig. 7*f*). He states that he made a direct observation of this occurrence: "Ich beobachtete diesen Fäden zufällig einige Zeit, und sah nun wie das spiralige Stück in ein Hin- und Her-schwankengerieth um schliesslich abzubrechen und als starre Spiralige hinweg zu schwimmen." This fact has been doubted, but the author has observed (Ellis (5)) an *intercalary* cell being detached from a thread of *Cladothrix dichotoma*, when it swam away in precisely the same manner as the detached fragment of *Beggiatoa alba* observed by Zopf. Finally, Zopf relates that the elongation of cocci and short rods into short filaments is sometimes followed by the assumption of the spiral shape.

Beggiatoa alba may therefore exist in the coccus, the bacillus, and the spirillum shapes; and each one of these may enter the zoogloea condition. Further, each kind may be motile or non-motile. The later development of these different forms is unknown, but there is no reason to doubt that, given the proper conditions, they develop once more into the filamentous condition.

Other Species of *Beggiatoa*.

Our knowledge of other species of this genus is somewhat scanty, and even their title to be included in the genus is doubtful.

Beggiatoa mirabilis (Cohn), 1865.

Literature.—Cohn (5), 1865; Warming, 1875; Engler, 1883; Bütschli (1), 1890; Kolkwitz (1), 1897; Hinze (1), 1901, and (2), 1902; Kolkwitz (10) 1918; Zuelzer, 1924; Bavendamm, 1924.

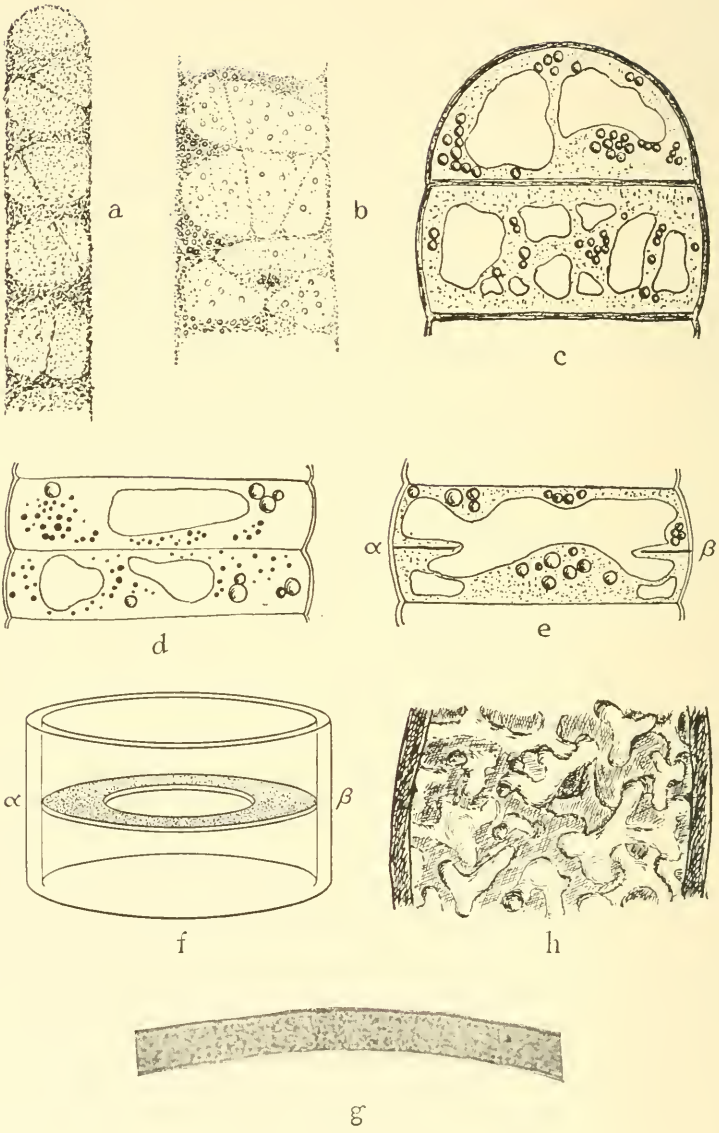


Fig. 8.

Description.—This remarkable organism is composed of threads varying in thickness from 15 to 45μ. In spite of its large size it has the same simple structure of cell as *Beggiatoa alba*. It differs, however, from that species in possessing a sharply defined external membrane and transverse walls similar to those of the genus *Bacillus*. Cohn depicts transverse walls and their existence has been confirmed by Hinze, who has described their structure in detail. Warming, however, depicts a

FIG. 8.—*Beggiatoa mirabilis* (Cohn), 1865, and *Beggiatoa mirabilis* (Warming).

a and *b* show parts of filaments of the organism which Warming described under this name. Transverse walls are not formed. × 500.

c-f show parts of filaments of the *Beggiatoa mirabilis* as figured by Cohn and Hinze.

c.—Shows the outer walls, the transverse membranes, the vacuoles and the sulphur globules. × 1200.

d (after Hinze).—Two cells separated by a delicate transverse wall: sulphur globules and bodies alleged by Hinze to be chromatin (see p. 182). × 1200.

e (after Hinze).—The first phase in the development of the transverse wall: formation of an annular ring. Seen in section at $\alpha\beta$. × 1200.

f.—Diagrammatic representation of the annular ring, shown in section in the preceding diagram.

g.—Photomicrograph of *Beggiatoa mirabilis* as described by Warming. From material from the South of England. × 500.

h.—*Beggiatoa mirabilis of Warming*.—A diagrammatic representation of the internal structure of a thread preserved in formalin. The plasma appears as an irregularly reticulate knotted mass. The nucleus is absent, and there are no transverse walls. At the time of writing living threads have not been obtainable, but there seems little doubt that in the living condition the structure of the plasma conforms to that shown in Fig. 6*b*. × 1200.

species by the name of *Beggiatoa mirabilis* (Fig. 8 *a* and *b*) which is essentially different from the organism figured by Cohn, in the complete absence of transverse walls. The thread appears to be roughly segmented, but this is not really the case, the fictitious appearance being caused by the occurrence of transverse bands of plasma at more or less regular intervals. In Fig. 8*g* is shown a photomicrograph of an organism found in the South of England (in material kindly sent to the author by Miss Nellie Carter) which corresponds in all essentials to the organism described by Warming. Viewed with a low

magnification the thread appears to be segmented, but with a higher magnification no signs of segmentation are seen. The disposition of the plasma is seen in Fig. 8*h*. Small projections of plasmatic material appear to jut out from the sides. The projections take the form of very irregular-shaped rods and point in all directions. There thus appears to be a single continuous vacuole along the whole length of the thread, occupying the space not filled up with the plasma. It is possible that transverse walls may be a feature in the further development of the threads, but such were not observed. Opportunities for studying the life-history of this organism have not yet occurred, but it is unlikely that it is identical with the *Beggiatoa mirabilis* of Cohn and of Hinze.

In Hinze's account of *Beggiatoa mirabilis* the appearance and development of the transverse walls are given. Its development begins with the formation of a ring-shaped flat projection round the inside of the cell (Fig. 8*e*). This is shown diagrammatically in Fig. 8*f*. This annular plate is pushed during development farther and farther inwards until the growing wall stretches across the whole cell. By intercalary growth the dividing cells attain the size of the mature cells. According to Hinze some of the cells die off and separation takes place at these points. Each of the separated threads then elongates afresh. They are naturally straight and of a somewhat stiff consistency, but become somewhat wavy from the unequal distribution of the numerous particles of dirt and other debris which settle on their surface.

Beggiatoa arachnoidea (Agardh), Rabenhorst (2), 1865.

Literature.—Agardh (2), 1827; Corda (1), 1835, (2), 1836; Kützing (2), 1843; Cohn (5), 1865; Warming, 1875 and 1876; Engler, 1883; Koppe, 1923; Bavendamm, 1924.

Description.—This organism was first mentioned by Agardh under the name *Oscillatoria arachnoidea*. Since that time several changes in nomenclature have occurred, and in 1865 Cohn transferred it to the genus in which it is now placed.

The threads are 5μ—6μ thick, and are divided into cellular segments by transverse bands of clear protoplasm placed at regular intervals. They form in the mass, thin, chalky-white slimy layers, of the texture of a spider's web. The segments are either as broad as they are long, or half as long as they are broad.

Habitat.—Found originally in the sulphur springs at Karlsbad. It occurs in waters, both fresh and salt, containing mud rich in H_2S.

Beggiatoa leptomitiformis (Meneghini), Trevisan (1), 1842.

Literature.—Meneghini (2), 1844; Massart, 1902.

Description.—This species is found in great numbers in various sulphur springs. The threads are not segmented.

They measure 1·8—2·5μ in thickness and form a chalky-white covering to the objects on which they settle.

Habitat.—Sulphur springs.

Genus 2.—*Thiothrix* (Winogradsky), 1888.

Founded by Winogradsky to designate a group of organisms very similar to, and frequently in association with, *Beggiatoa*. The genus differs from *Beggiatoa* in the following respects:—

1. Absence of free movement.
2. Presence of an anchoring slimy organ of attachment.
3. Development of a slime sheath.
4. Formation of "conidia" at the free end of the threads. These are thrust out of the sheath, and after a slow, creeping, independent movement, attach themselves to objects in the water, where they form new threads.

It is not always possible to distinguish these two general for *Beggiatoa alba* sometimes develops excessive slime, when it becomes stationary, and resembles *Thiothrix*. Again, another species, *Beggiatoa arachnoidea*, possesses an anchoring organ of attachment, and thus also resembles *Thiothrix*. But slime development resulting in an enveloping hollow sheath is a normal feature in the life-history of *Thiothrix*. This sheath

hardens and becomes detached from the remainder of the organism, forming a covering which encloses the living part of the cell. Finally, although short lengths of *Beggiatoa* do not show any difference in thickness at different parts of the thread, longer filaments show differences which may be pronounced. In *Thiothrix* the presence of a fixed hardened slime sheath has some influence in determining the thickness, since normally the apex, where the slime is softer, is thicker than the base, where the slime is firmer.

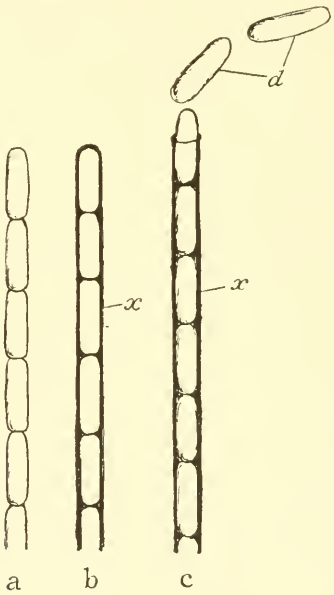


FIG. 9.—*Thiothrix Rod-Gonidia* × 1000.

a.—Young thread before active slime formation.
b.—Slightly older thread, in which slime formation has commenced. Slime shown at *x*.
c.—Later stage. Slime has hardened into a sheath.
Rod-Gonidia (*d*) are in process of expulsion from open apex of sheath.

Description.—Colourless sulphur-containing threads, non-motile in mature form, and surrounded by an enveloping sheath of hardened slime. Usually attached by a slimy anchoring organ to various objects in the water. Distinction between base and apex much more marked than in *Beggiatoa*, and the middle portion may occasionally be thicker than

either the base or the apex. The thread readily breaks up into more or less equal segments which for a time are held together by connecting bands of slime. The whole surface of the cells is covered by a layer of slime which subsequently hardens, and forms round the cells an enveloping hollow tubular sheath. With further growth the apex of the line of cells inside the tubular sheath is pushed out. Then each cell as it wins clear of the top of the sheath becomes separated and moves away. There is thus a steady emergence of cells from the sheath, pushed from behind by the pressure of growth. In some cases the liberated cells develop motility and swim slowly away. The name *rod-gonidium* is usually given to a cell of this kind. After swimming for a short time it comes to rest on some object and forms a new thread (Fig. 9).

Note on the Terms "Conidium" and "Rod-Gonidium."

The use of these terms is incorrect in the sense in which they are employed above, as it implies that they are special organs of asexual reproduction. The method of reproduction in *Thiothrix* is one of simple fission, but it is complicated by the circumstance of the fission taking place inside a sheath of slime. In essentials it consists merely in fragments of a thread being successively detached from the apex.

Thiothrix nivea (Rabenhorst), Winogradsky, 1888.

Literature.—Pollini, 1817; Oerstedt (2), 1844; Kützing (2), 1843; Rabenhorst (2), 1865; Corsini, 1905; Swellengrebel, 1909; Keil, 1912; Bavendamm, 1924.

Synonyms.—*Conferva alba* (Pollini); *Leptomitus* (Agardh); *Leucothrix Mucor* (Oerstedt); *Hygrocrocis nivea* (Kützing); *Beggiatoa nivea* (Rabenhorst); *Thiothrix tenuis* and *Thiothrix tenuissima* (Winogradsky, Migula, Buchanan, and Bavendamm).

Description.—Bavendamm suggests that this species was described as far back as 1817 by Pollini under the name *Conferva alba*, and at various times, as is shown by the literature quoted, attention has been called to it by various investigators. Rabenhorst in 1865 gave to it the name *Beggiatoa nivea*, which was subsequently (1889) changed by Winogradsky to *Thiothrix*

nivea. The last-named investigator divided one species into three on the sole basis of differences in the thickness of the threads. As all these different forms were found in the same medium, and as numerous intermediates were also present, the subdivision of the species is not warranted.

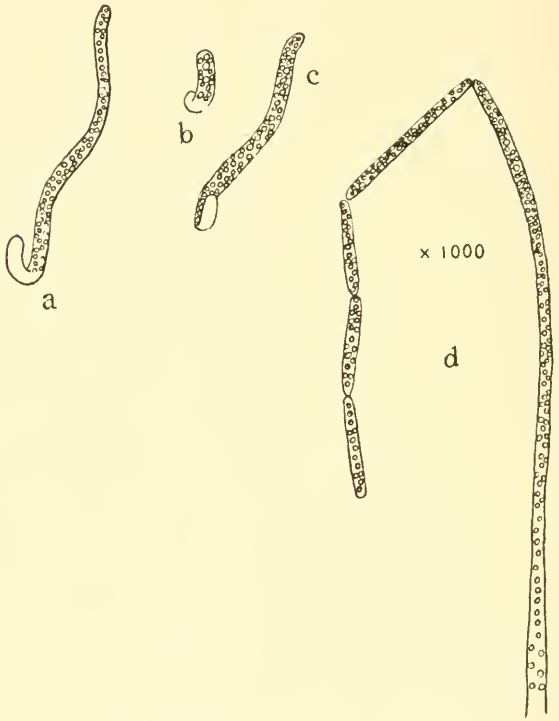


FIG. 10.—*Thiothrix nivea*. × 1000.

a, b, c.—*Thiothrix nivea*. Shows young filaments with slime cushions at the base of each.

d.—*Thiothrix tenuis*. Filament from the free end of which "conidia" are in process of liberation. Four of these are shown at point of separation from the parent filament.

NOTE ON THE VARIANT FORMS OF *THIOTHRIX NIVEA*.

The following figures are supplied by Winogradsky as the distinguishing marks of the three "species" mentioned by him:—

	Base.	Middle.	Apex.
Thiothrix nivea	2—2·5μ	1·7μ	1·4—1·5μ
,, *tenuis*	All threads of uniform thickness and = 1—1·1μ		
,, *tenuissima*	All threads of uniform thickness and = 0·3—0·5μ		

As all these forms may be found in the same microscope-field, the inference that may be drawn is that threads of *Thiothrix* are of uniform thickness when very young, and that they show inequality of thickness when they further develop. It appears as though Winogradsky had assigned different specific names to different stages in the development of one organism.

Habitat of Thiothrix nivea.—Found in sulphur springs and in waters, both fresh and salt, which contain sulphuretted hydrogen in solution.

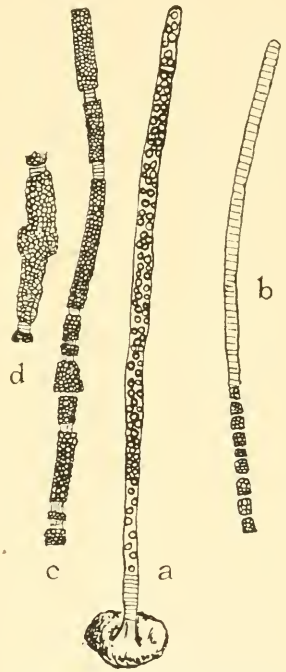


FIG. 11.—*Thiothrix marina* (Molisch, 1907).

THIOTHRIX ANNULATA (Molisch).

Literature.—(Molisch (5).)

Description.—The threads are attached by a well-developed slime anchor (about 3·5μ in thickness). The young colonies are composed of short, sulphur-filled threads, all in attachment to the same slime anchor (Fig. 11*a*). After further growth a long thread is formed which is at first unicellular, but later is segmented by transverse septa. In Fig. 11*b* is shown a still older thread in which the upper part is septate, but free from sulphur. Very old threads may attain a thickness of 7μ, and exhibit knotty thickenings separated by ring-like, constricted, sulphur-free areas (Fig. 11*c*). Fragments break off at the constricted parts and a portion of such a fragment

is shown in Fig. 11*d*. The threads, except in very young stages, are not uniformly thick. The following measurements were obtained from a typical example: base 2μ, middle 3—4μ, and apex 1·8μ. The septa were 1μ apart.

Habitat.—Found by Molisch in sea-water from Trieste containing decomposing Algæ; and by Bavendamm in similar material from the neighbourhood of Berlin, and from the briny wells of Sperenberg (Brandenburg, Prussia).

Thiothrix marina (Molisch), 1907.

Literature.—Molisch (5), 1907; Bavendamm, 1924.

Description.—This species forms a white pellicle on the same material as that on which *Th. annulata* thrives. Bavendamm considers it to be identical with *Th. tenuis*, that is, *Th. nivea*.

The threads are relatively long and thin, being 0·8—1·3μ thick and 130—300μ, sometimes up to 500μ, long.

Thiothrix violacea (Ellis).

In all systems of classification of the sulphur bacteria, the first division is based on the presence or absence of colour. This procedure is satisfactory in the majority of cases, as the distinction is felt to be a natural one, and its utility is obvious. The author has, however, found in Possil Marsh, near Glasgow, a coloured *Thiothrix*. A difficulty in the allocation of this species thus arises. In its structure and life-history it is a typical *Thiothrix*, and yet being coloured, it should find a place in the *Rhodo-thiobacteria*. It is proposed to place this organism provisionally in the genus *Thiothrix*, on account of its obvious relationship.

Description.—Violet coloured, uniformly thick filaments, showing sheath-formation. Reproduction by fission, and possibly by endospores. The threads are approximately 3μ thick and are found attached basally to various objects in the bed of a stream containing domestic sewage. There is no special anchoring cushion of slime. Transverse walls (of slime) are not visible in young threads, but they appear in old threads, and also in threads that have lost their

sulphur contents (Fig. 12*a*). In older threads a sheath of slime is formed from the outermost layers of the cells, which

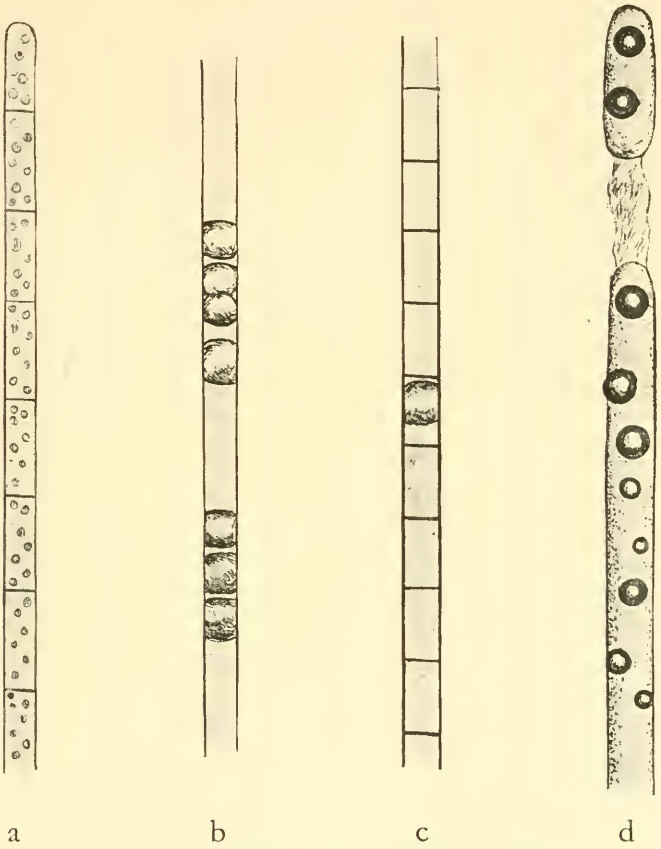


FIG. 12.—*Thiothrix violacea* (Ellis). × 1200.

a.—Fragment of old thread made up of the hardened slime sheath, the transverse bands of slime, and unidentified particles.

b.—Fragment of old thread containing seven round bodies, possibly endospores. Previously formed transverse bands of slime have been swept away by growth in the cells within the hardened sheath.

c.—Fragment of a thread with an open end and transverse bands of slime.

d.—Thread showing so-called "conidia" formation. The "conidium" is attached to the parent thread by a band of slime, which can be seen by treatment with carbol-fuchsin.

subsequently hardens and forms a hollow tube containing the cells in single row formation. In Fig. 12 *b* and *c*, are shown

two old threads, of which *b* is a hollow sheath of hardened slime and containing seven of the objects which may be endospores; whilst *c* is a similar thread, but in this the transverse bands of hardened slime are also present. It also contains one of the objects that may be endospores.

The plasma is homogeneous, with the sulphur globules and pigment uniformly distributed. In older threads from which the sulphur globules have disappeared a large number of small granules are seen. The nature of these has not been determined.

Methods of Reproduction.

(A) Reproduction by *fission* is the common method. It may take place terminally, or it may be intercalary. Intercalary growth is found only in young threads. In older threads only the tip goes on dividing, and short lengths of the filament are successively broken off. These are often named "conidia" or "rod-gonidia," which are misnomers, for the difference between such reproduction and fission is one of degree not of kind (see also note on p. 107). In Fig. 12*d* is shown a young thread in process of liberating a short length from its tip. Connection with the parent thread is still maintained by a band of slime. The same process is in force even when the filament is enveloped by a sheath, for the top of the sheath is open, and the cells are pushed out of the opening by pressure from behind.

(B) *Endospores.*—In older filaments a number of bodies are occasionally seen which may be *endospores* (Fig. 12 *b* and *c*). They are roughly cylindrical-elliptical, and approximately of equal size (about 3μ). They were found in old threads of which nothing remained except the sheath. For lack of opportunity, the germination of these structures was not followed so that their spore nature has still to be proved.

Habitat.—Found in a running stream containing domestic sewage, near Glasgow.

Note.—The investigation of this organism was unfortunately cut short by its disappearance following the introduction of sanitary measures, which diverted the course of the sewage.

Genus 3.—*Thioploca* (Lauterborn), 1907.

Literature.—Lauterborn (5), 1907; Wislouch (1), 1912; Koppe, 1923; Bavendamm, 1924.

A well-defined genus living in the calcareous mud of shallow marine waters.

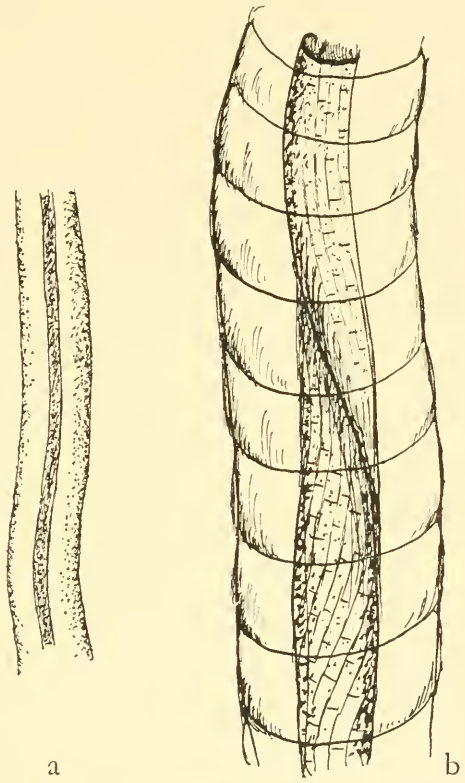


Fig. 13.—*Thioploca Schmidlei* (Lauterborn). × 5000.

Description of genus.—Colonial motile threads of the *Beggiatoa* type bound together in a common envelope of slimy matter. Each colony has the appearance of a rope of slime, but the individual movements of the contained threads do not result in their separation from the colony. The outer surface of the slime rope is covered with particles of mud and gritty matter, and is constricted at regular intervals (Fig. 13*b*),

each constriction being ring-shaped and transverse. The threads are septate, and their ends often pointed.

Wislouch states that the members of this genus are slightly blue in colour, due to the presence of phycocyan and chlorophyll. It is probably physiologically intermediate between the *Cyanophyceæ* and the *Leuco-thiobacteria*, but it is included in this group for the sake of completeness.

Thioploca Schmidlei (Lauterborn), 1907.

Literature.—Lauterborn (5), 1907 ; Koppe, 1923.

Description.—Each "rope" may extend to 3—4 centimetres, and may itself be one of a number of strands forming a thicker rope. The thickness of such a "rope" is 50—60μ, and within it the closely adpressed threads at its centre exhibit gentle gliding movements passing over one another, but they do not leave the "rope" in their movements. Also there appears to be some directive force in the community as a whole, for the movements of two neighbourhood threads are always in opposite directions. Sculpturing effects are produced on the surface of the "ropes" owing to the incrustation of mineral matter from the surrounding water. At certain periods, or under certain conditions, the slime opens out and from it emerge threads, which swim away and doubtless form the nucleus of new colonies, although their development has not been investigated. Reproduction is by fission. Lauterborn also observed the intertwining, after separation, of the two sections of a dividing thread.

The threads are septate, the length of each segment being $1\frac{1}{2}$ times its breadth. The threads in a young colony, according to Lauterborn's figures, do not show septation. The sulphur granules are so numerous and small that the threads appear black under the microscope.

Habitat.—Found in calcareous mud below 15—20 metres depth either in marine, or brackish, or fresh water. Recorded from Ermatingen; from the neighbourhood of Solingen, in a bay of the Rhine; from near Strassburg; from the Neva; and from the neighbourhood of Königsberg.

Thioploca ingrica (Wislouch), 1912.

Literature.—Wislouch (1), 1912; Kolkwitz (7), 1912; Koppe, 1923; Bavendamm, 1924.

Description.—The rope-like masses which constitute this species are white in colour, are about 1 centimetre long, and are composed of threads rich in sulphur. The threads creep along both directions of the longitudinal axis. Each rope contains from ten to twenty threads, some with blunt, others with pointed ends.

Habitat.—Same as preceding species. Has been found in Leningrad, in a harbour in the Gulf of Dantzig (Frisches Haff), and in the Boden See (Switzerland).

The differences between the two species of this genus may be tabulated as follows :—

	Th. Schmidtei.	*Th. ingrica.*
Lengths of slimy masses	A few cms.	Up to 1 cm.
Thickness of slime envelope	50—60μ	Up to 80μ
Thickness of thread	5—9μ	2—4·5μ
Length of cells	5—14μ	1·3—8μ

Recently two species of this genus have been described by Koppe, and named respectively *Thioploca minima* and *Thioploca mixta*. As the distinction between them and the two preceding species is confined to a difference in dimensions only, and as they are found mixed with *Thioploca ingrica*, and as in *Thioploca mixta* different thicknesses of thread are found in the same colonies, we are entitled to conclude that these latest additions are merely variant forms of *Thioploca ingrica*.

CHAPTER VII.

LEUCO-THIOBACTERIA (COLOURLESS SULPHUR BACTERIA).

Family 2. *Achromatiaceæ*. Genus 1. *Achromatium*; Genus 2. *Thiophysa*; Genus 3. *Thiosphærella*; Genus 4. *Thiovulum*.
Family 3. *Thiospirillaceæ*. Genus 1. *Thiospirillum*.
Family 4. *Thiobacillaceæ*. Genus 1. *Thiobacillus*; Genus 2. *Thiopseudomonas*.

Family 2.—*Achromatiaceæ*.

Free, motile, and usually spherical or globoid. In one genus (*Thiovulum*) cilia have been demonstrated. Reproduction by fission; and in one genus by zoospores.

Genus 1.—*Achromatium* (Schewiakoff), 1893.

Literature.—Warming, 1876; Schewiakoff, 1893; Frenzel, 1897; Lauterborn (1), 1898, and (6), 1913; Massart, 1902; Zacharias (2), 1903; West and Griffiths (1), 1912, and (2), 1913; Kolkwitz (3), 1909; Virieux (1), 1912, and (2), 1913.

Free, motile, and spherical, or spheroidal or cylindrical-elliptical. Reproduction by fission and by zoospores.

Achromatium oxaliferum (Schewiakoff), 1893.

Literature.—See above.

Habitat.—Widely distributed in most European countries, and probably outside Europe.

Description.—The cell is ellipsoidal and normally appears full of dark spherical bodies, as shown in Fig. 14A. The movement is very slow, and consists, usually, of a forward or

backward motion with an occasional rotation on its longitudinal axis. No organs of movement have been found, although Zacharias states that young individuals possess a clearly visible cilium as long as the organism itself. This has not been confirmed.

Although probably first mentioned by Warming in 1875, the first descriptive account of the organism was given by Schewiakoff in 1893. He described and named *Achromatium oxaliferum*, which is considered to be identical with the organism subsequently described by Frenzel (1897) under the name of *Modderula Hartwigi*, and by West and Griffiths (1909) under the name of *Hillhousia mirabilis*. In the works of the earlier writers (Schewiakoff, Frenzel, Lauterborn) an attempt is made to distinguish between the peripheral layer (Rindenschicht) and the central body (Centralkörper), obviously under the influence of Bütschli's now discredited view of the structure of the bacterial cell, and so the plasma is described as being composed of two layers, a central with larger, and a peripheral with smaller, meshes. This marked distinction between the size of the meshes has not been observed by later writers as Virieux, and West and Griffiths, who have examined the structure of the cells with greater accuracy. Dr. B. M. Griffiths regards *Hillhousia* as specifically distinct from Schewiakoff's *Achromatium oxaliferum* and Frenzel's *Modderula Hartwigi*, because in it there is no distinction between a peripheral and a central plasma. This distinction, however, does not occur in any member of the sulphur bacteria nor in any one of their immediate allies. The distinction apparently did not exist in the organism examined by Schewiakoff and by Frenzel. It is noteworthy that, although the organism named *Modderula* was stated to be quite common, no organism of the bacterial group containing a differentiated plasma has since been found. If, therefore, we take the view that *Modderula* (including *Achromatium*) does not possess a differentiated plasma, the sole distinction between *Hillhousia* and this organism vanishes, and on the ground of priority the name *Achromatium oxaliferum* must be held to cover all three generic names (see Fig. 51 *a* and *b*, and pp. 182-4).

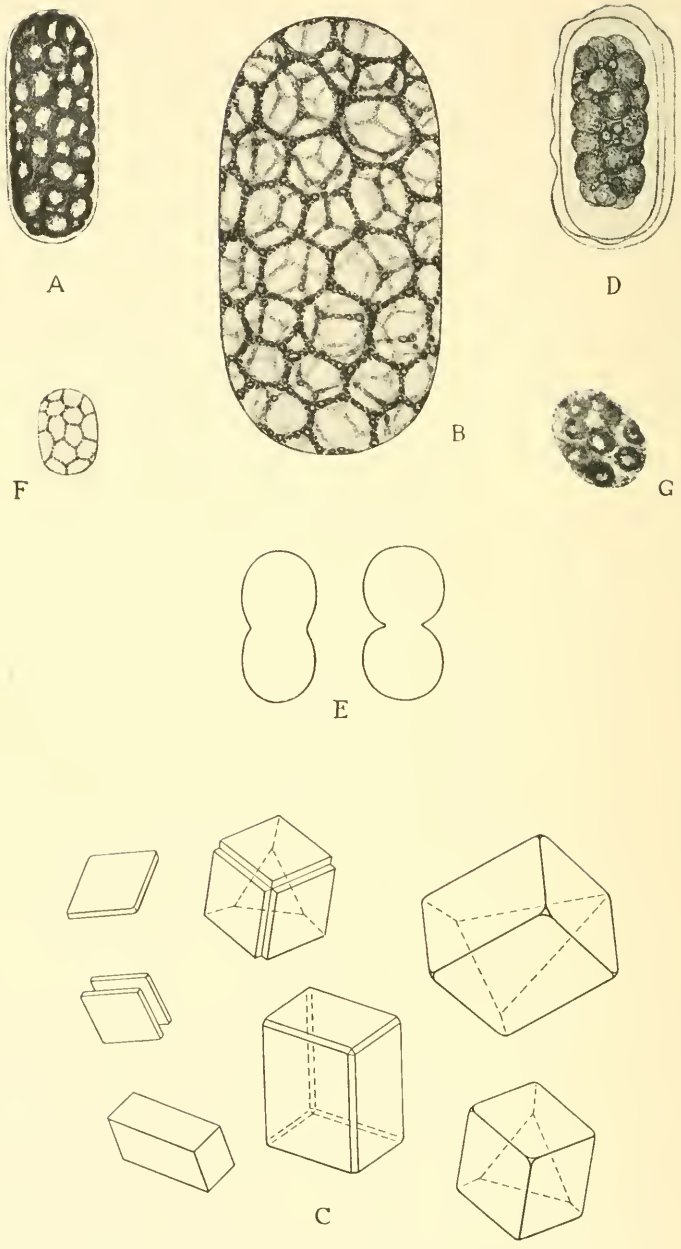


FIG. 14.

Marked differences in size are noted in this organism:—

Schewiakoff . . .	$15\mu \times 9$—22μ.
Frenzel	9—$15\mu \times 6$—33μ.
West and Griffiths . .	40—$60\mu \times 20$—33μ.
Nadson	Up to 102μ in length.

The smallest appears to have been that recorded by Bavendamm, which was only 3μ in length.

Achromatium supplies another illustration of the fact that all widely distributed bacteria exhibit a wide range of dimensions, and that within the extremes of this range are

FIG. 14.—*Hillhousia*. (West and Griffiths.)

A.—Normal aspect of living specimen of *Hillhousia mirabilis*. × 500. The cell is filled with globules of calcium carbonate.

B.—*H. mirabilis* after removal of calcium carbonate by dilute acetic acid. The conspicuous granules are grains of sulphur. × 850.

C.—Rhombic crystals of calcite, obtained by allowing the organism to dry, and then irrigating with distilled water. × 850.

D.—After treatment for 15 minutes with 5 per cent. phenol. Shows lamellose cell wall.

E.—Diagrammatic representation of two stages in the division of *H. mirabilis*.

F.—Cell after removal of cell contents.

G.—Photomicrograph of *H. mirabilis* from material kindly sent to the author by Dr. B. M. Griffiths. × 450.

a-f are taken from West and Griffiths' paper (see Bibliography).

a multitude of intermediate forms. Hence one specific name should cover all that fall within the range. A new specific name is called for on the score of differences in size only when a distinct gap occurs in the grading. In plastic organisms like the sulphur bacteria, a variation in the size of the different individuals in a culture has no more genetic significance than the variation of the number of sulphur globules in the cells.

METHODS OF REPRODUCTION.

(A) *Fission*.—Two stages in division are shown diagrammatically in Fig. 14E. This process has not been followed in detail.

Virieux claims that nuclear material is present as chromatin granules. If so, their disposition during cell division should be a matter of interest. The method of division is that which is common to the great majority of the sulphur bacteria. The plasma separates without the previous formation in it of a definite cell wall.

(B) *Zoospores.*—The cell gradually expands, finally bursting its membrane, and liberating a number of zoospores that, during this period of expansion, have formed inside. Each zoospore resembles the adult in structure, and differs only in

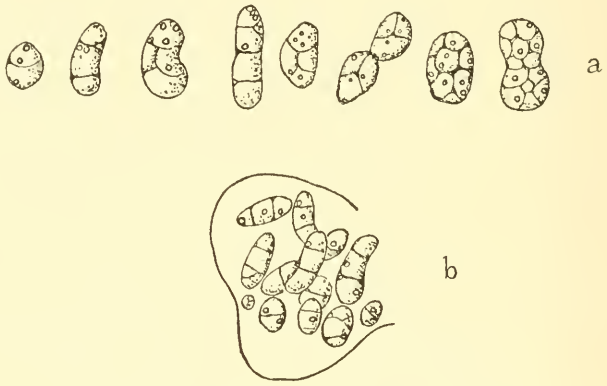


FIG. 15.—*Achromatium oxaliferum.* (After Virieux.)

a.—Zoospores. They show the diversity of form and characteristic alveolar structure of this organism. × 1500.

b.—Liberation of zoospores from the mother cell. × 1000. (For further explanation see text.)

showing some diversity in shape (Fig. 15). It measures 4—6μ in length, although some reach 10—11μ. It exhibits the alveolar structure characteristic of the adult, and in each are 2—3 *granules* and a few *corpuscles* (see p. 184). Cilia are not developed. The zoospores on liberation increase without any further change to the size of the adult cell.

Habitat.—Present in water, both fresh and salt, in which H_2S is present as the result of vegetable decomposition, and in which also calcium salts are abundant. The organism has been found in various places in Germany (Lauterborn, Frenzel, Bavendamm, etc.); in Belgium (Massart); in England (West

and Griffiths); Austria and Czecho-Slovakia (Molisch). It has also been recorded from Ireland, Denmark, Russia, and South Africa. Its presence has been frequently noted in lake deposits of vegetable debris and diatomaceous muds; and in vegetable debris in marshes. Distribution is probably world-wide.

Achromatium mobile (Lauterborn), 1915.

Syn. *Microspira vacillans* (Gicklhorn).

Literature.—Lauterborn (7), 1915; Gicklhorn (1), 1920; Bersa (1), 1920; Utermöhl, 1923; Koppe, 1923.

Description.—The general appearance of this organism is shown in Fig. 16. Lauterborn placed it in the genus *Achromatium*, but later writers have considered its differences sufficiently marked to merit the formation of a new genus. Utermöhl, and Koppe gave it the generic name of *Macromonas*, and what is apparently the same organism is described by Gicklhorn (1) under the name of *Microspira vacillans*. Pending a more comprehensive investigation the organism is best retained in the genus in which it was placed by Lauterborn.

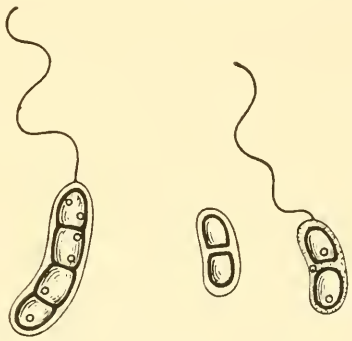


Fig. 16.—*Microspira vacillans*. × 700.

Microspira vacillans (*as described by Gicklhorn*).*

The organism is a single ellipsoidal cell, somewhat bent in the middle (Fig. 16), measuring 12—30μ × 8—14μ. It contains numerous globules of sulphur, and in addition two or three large bodies showing a bluish tinge. These, according

* This writer's work was not available, and the statement about the composition of these bodies is made on the strength of the title of Bersa's work *Über das Vorkommen von kohlensaurem Kalk in einer Gruppe von Schwefelbakterien*.

to Bersa, are composed of calcium carbonate. There is a single large polar cilium, which is plainly visible without special preparation, and which is as long as, or even longer, than the cell. Cell-division takes place by simple fission following upon a slight elongation of the cell, which during the process does not come to rest. The stages in division are the same as in *Achromatium oxaliferum*. After fission has taken place the two daughter cells are connected for a period by a transverse band of hyaline material. The cells gradually draw apart, and complete separation is effected. The number of small globules of sulphur is increased when sulphuretted hydrogen is added to the medium.

Habitat.—Found in foul mud samples from the University garden in Graz (Austria).

Genus 2.—*Thiophysa* (Hinze).

Literature.—Hinze (3), 1903; Nadson (5), 1914.

A genus of two species. The cells are globular or ovoid, colourless, and motile. The movement, which is slow, is one of translation and of rotation. Cilia have not been found. The cells contain a peripherally placed plasma, and a large central vacuole. Division takes place by fission, following a slight preparatory elongation. Both uni- and diplo-cocci are common.

Thiophysa volutans (Hinze).

Literature.—Hinze (3), 1903; Nadson (5), 1914; Kolkwitz (10), 1918.

Description.—This species was found in a sulphur spring in the Gulf of Naples, near Castellamare. The floor containing the organism was made up of fine calcareous sand, and smelt strongly of H_2S. The calcium does not appear to penetrate the cell membrane. Diameter of cells, 7—18μ; maximum = 28·9μ long, and 17·9μ broad (Fig. 17).

It has also been found in the brine ditches at Artern (Saxony), and at the sea-bottom near Hapsalu in Esthonia.

Thiophysa macrophysa (Nadson).

Literature.—Nadson (5) 1914.

Description. — This species is distinguished from the preceding by its larger average size. The diameter of cell is 21—40μ. It is, therefore, a much larger organism than the preceding, and, in addition, there is a physiological distinction, for whilst the *Th. volutans* is confined to marine waters, this species flourishes only in a medium containing much less sodium chloride, namely $\frac{1}{2}$ per cent.

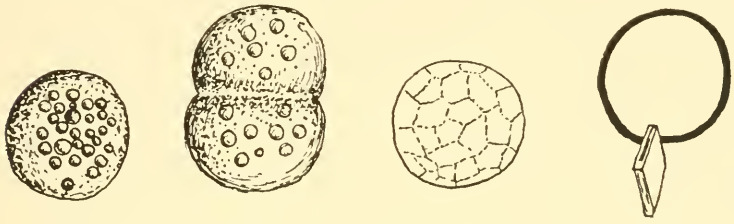


Fig. 17.—*Thiophysa volutans.* × 500.

Genus 3.—*Thiosphærella* (Nadson).

Literature.—Nadson (5), 1914; Bavendamm, 1924.

One species, *Thiosphærella amylifera.* The following description is translated from Bavendamm's work, as Nadson's work was not available. Cells round, often ellipsoidal, 6μ long, and 4·8μ broad. The membrane is relatively thick, dense, and highly refractive, and surrounding it is a colourless slime layer. The protoplasm is normally not quite colourless, but has a steel-grey tint. In the plasma are sulphur globules of various sizes, and, in addition, a substance of a starchy nature, which colours a dark red or violet when treated with iodine. The cells possess a slow movement, similar to that of *Achromatium.* Reproduction is by fission (Fig. 17 *a* and *b*).

Genus 4.—*Thiovulum* (Hinze).

Literature.—Warming, 1876; Migula (3), 1900; Molisch (5), 1912; Hinze (6), Lauterborn (7), 1915; Bavendamm, 1924.

Description.—The cells are ellipsoidal, sometimes pointed, or considerably flattened. The membrane is sharply defined, and the plasma is made up of delicate strings which traverse the whole cell. In some the plasma may be massed all at one end, leaving a large vacuole in the remainder of the cell. The sulphur globules are soluble in 90 per cent. alcohol, and insoluble in hydrochloric, nitric, and acetic acids. Also, the peripheral plasma contains dull green, round, or oval, or sometimes angular, plates of varying sizes. The poorer the cells become in their content of sulphur, the more numerous are these plates. They are regarded as some form of reserve material. The cilia are numerous, and peritrich, each about a third of the length of the cell, and 0·7μ in thickness. Reproduction is by transverse fission.

Hinze distinguishes two species :—

Thiovulum majus, between 11 × 9μ and 18 × 17μ.
,, *minus* ,, 9·6 × 7·2μ and 11 × 9μ.

Thiovulum Mülleri (Warming).

Literature.—Warming, 1876 ; Migula (3), 1900 ; Molisch (5), 1912 ; Hinze (6), 1913 ; Bavendamm, 1924.

Description.—In 1876 Warming described and depicted an organism, which he named *Monas Mülleri*, that belongs indubitably to the same group as *Thiovulum*. Thus the generic name *Monas* * has prior claim, but as this name is already applied to a genus of one of the Flagellate groups, it may conveniently be ignored.

The cells are ellipsoidal or globular (Fig. 18). The size varies from 6·3μ to 12·8μ in length, and from 5·6μ to 15μ in breadth. Hinze has recommended that the species *Thiovulum Mülleri* be limited to such members as are within the dimensions 4·9—12μ in length. The suggestion is not to be recommended, for there is obviously no specific distinction between the members of these dimensions and those immediately shorter or

* *Monas.*—The term is applied to a division of Flagellates, a group which is divided according to the number and position of the flagella or cilia. The genus *Monas* is distinguished by the possession of one flagellum.

longer, and there is more to be lost than gained by unnecessarily adding to the number of species.

The cells show quick, agile movements and appear to be devoid of cilia. Reproduction is by longitudinal fission. The intimate structure of the cell is given on page 185. Hinze

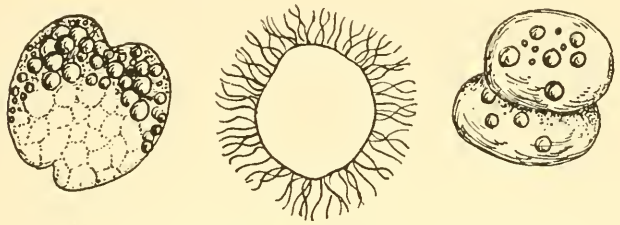


FIG. 18.—*Thiovulum Mülleri.* × 2000.

claims that a certain organ in the cell which is colourless, but which possesses a centrum that can be stained with Delafield's hæmatoxylin, is a nucleus, and that cell division is accompanied by the division of this organ (see Fig. 19). He also states that when the H_2S supply is scarce, starch or an amyloid compound is stored in the cell, probably as a food reserve. An interesting feature in Hinze's work is his observation that the cells are still in possession of a large number of sulphur granules when, after an active life, they have come to rest and apparently died. Thus there are other determinants besides sulphur in the metabolism of this organism.

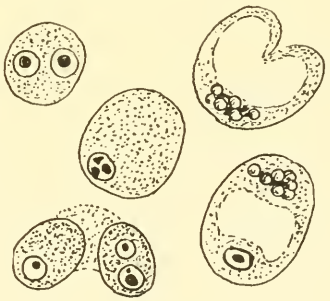


FIG. 19.—*Thiovulum Mülleri.* × 1500.

Habitat. — Found on the Danish coast, in various parts of Germany, in Austria, and in the Gulf of Naples. It is probably an organism of universal distribution. It lives in water above mud rich in sulphuretted hydrogen, and thrives particularly well in salt water, although it is also found in fresh waters.

Note on Thiovulum.—The discussion of this organism should more appropriately appear in a work on the *Flagellates*, but place is given to it in this work because the limits of the group are defined by a physiological trait, namely the sulphur metabolism. It is obvious, however, that the sulphur-organisms are polyphyletic and that whilst the majority belong to the *Schizophytes*, some, like *Thiovulum*, cannot be classed as Bacteria.

Family 3.—*Thiospirillaceæ.*

Free motile, colourless cells, containing sulphur. Division by transverse fission.

As the spiral sulphur bacteria differ from the *Spirillaceæ* of the *Eubacteria* only in the inclusion of sulphur granules, the term *Thiospirillum* used by Omelianski is preferable to the term *Thiospira* used by Wislouch. The former has also a claim on the ground of priority. Following the nomenclature adopted in this book the following generic terms may be distinguished :—

Spirillum.—Colourless spiral bacteria : sulphur absent.

Thiospirillum. — Colourless spiral bacteria, containing sulphur.

Rhodothiospirillum.—Coloured spiral bacteria, containing sulphur.

Rhodospirillum. — Coloured spiral bacteria ; sulphur absent.

The prefix "*Rhodo*" was first used by Molisch in his *Purpurbakterien* to designate purple coloured bacteria, and included the sulphur-containing and the sulphur-free forms.

Genus 1.—*Thiospirillum* (Omelianski).

Literature.—Omelianski (2), 1905 ; Molisch (2), 1912 ; Wislouch (2), 1914 ; Perfiljeff, 1923 ; Bavendamm, 1924.

Colourless, spirally wound, with polar cilia. Division by transverse fission, as in all Spirilla.

Thiospirillum Winogradskii (Omelianski).

Literature.—Omelianski (2), 1905; Molisch (5), 1912.

This species was found in 1903 at the bottom of a high glass vessel which had been filled with mud and water, from the limans near Odessa, and to which some lime had been added. It is a large, motile, colourless, or almost colourless, spirillum, about 40μ long and 3·5μ thick, as sketched by Omelianski (Fig. 20). The normal form has one spiral turn but some show two such turns. The single cilium, which is probably compound, is polar.

Habitat.—Found in Russia (Omelianski); Austria (Molisch); and Germany (Kolkwitz). It is common in water, both fresh and salt, containing hydrogen sulphide.

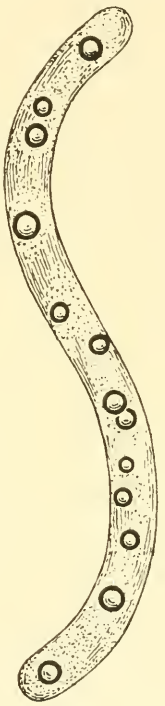


Fig. 20.—*Thiospirillum Winogradskii* (Omelianski). × 2500.

Thiospirillum granulatum (Molisch).

Literature.—Molisch (2), 1912.

An organism found in an artificial culture containing fresh water, mud, gypsum, and roots of Elodea. This is a doubtful species, as it was found in the same medium which contained *Beggiatoa alba* and *Cladothrix*, both of which are pleomorphic, and in some stages have a spiral form. It is provisionally included here. The length is given as 21—40μ and its thickness 2—3·5μ.

Thiospirillum bipunctatum (Molisch).

Literature.—Molisch (2), 1912; Wislouch (2), 1914.

A marine organism showing a half, or a complete turn, with a clear zone in the middle. The sulphur granules are located in this middle portion. There is one cilium at each pole. Volutin is present. Length of thread, 6·6—10μ, and thickness 1·9—2·4μ (Fig. 21).

Habitat.—Found in mud from Trieste, in which hydrogen sulphide was present. Also in mud from the harbour of Reval (Esthonia).

Thiospirillum agilissimum (Gicklhorn), Ellis.

Literature.—Gicklhorn (1), 1920; Bavendamm, 1924.

The generic name *Spirillum* was given to this organism by Gicklhorn, and, following Wislouch, changed by Bavendamm to *Thiospira.*

A small S-shaped, or comma-shaped, spirillum, found in mud samples from a pond at Graz (Poland). It measures 10μ in length and about 1·8—2·0μ in thickness. Gicklhorn

Fig. 21.—*Thiospirillum bipunctatum* (Molisch).

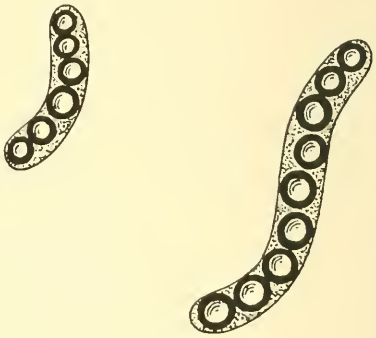


Fig. 22.—*Thiospirillum agilissimum* (Gicklhorn), Ellis. × 3000.

describes it as one of the smallest forms of sulphur-spirilla. Its motility is very great. The sulphur droplets are pressed close together, and number 4 to 12 in each cell. The membrane is very delicate (Fig. 22).

The introduction of hydrogen sulphide into the medium containing the organism had no apparent effect in promoting growth.

Thiospirillum agilis (Kolkwitz), Ellis.

Literature.—Kolkwitz (3), 1909; Lauterborn (7), 1915; Koppe, 1923; Bavendamm, 1924.

This organism was found in mud rich in hydrogen sulphide. Its characteristics are given as follows: Thickness 1μ, one

or two windings, width of winding 6μ, sulphur globules disposed in a single line.

Habitat.—Found in various parts of Germany.

Note.—It is doubtful whether this organism is sufficiently defined from the above description to justify giving it a specific name.

Thiospirillum elongata.—Perfiljeff, 1923.

Literature.—Perfiljeff, 1923; Bavendamm, 1924.

Bavendamm gives the following description. Slender cells with steep windings, and pointed ends. Dimensions 1·2—1·5μ thick (in the middle) and 12—28μ long. The ends are filled with volutin granules. Two polar cilia. Membrane with spiral thickening.

Habitat.—Found in fresh water overlying mud rich in hydrogen sulphide, near Leningrad.

Note on the Sulphur spirilla.—It is unfortunate that practically nothing is known of the life-histories of these interesting forms, and specific names have been given almost entirely from the appearance presented by the adult organisms.

Family 4.—*Thiobacillaceæ.*

Free, colourless, motile or non-motile cells. Division by transverse fission. The term *Bacillus* in Migula's classification is used to designate motile, rod-shaped cells, and the term *Bacterium* is used for similar cells that are non-motile. It is doubtful, however, whether the distinction can be maintained. Five species of the genus *Bacterium* were chosen at random, and in all it was found possible to induce movement (see Ellis (3)). It is, therefore, extremely probable that the motility or non-motility of these organisms is conditioned by the nature of the environment, and that the two terms apply to the same organisms. In this work the term *Bacterium* is not used with generic significance, and the term *Bacillus* is used for both the motile and the non-motile rod-shaped organisms. There is another reason for discarding the word *Bacterium*. Its proper use in the English language is obviously as the singular of the word *Bacteria*, and it would therefore be better to restrict its

use to designate a single member of the group of *Bacteria*. The word *Bacterie* which often appears in German literature as the singular of *Bacterien* (or *Bakterien*) is not so suitable.

The following terms are used in this work :—

Thiobacillus. Short rods, colourless, and with sulphur contents.
Rhodobacillus. ,, coloured, and no ,, ,,
Rhodothiobacillus. ,, coloured, and with ,, ,,

If the ciliation is polar instead of peritrich the term *Pseudomonas* must replace that of *Bacillus*, with the same meaning as in Migula's classification. Thus there are theoretically six genera, four coloured and two colourless. In this section only the genera *Thiobacillus* and *Thiopseudomonas* are considered. Both *Thiobacillus* and *Rhodobacillus* are already in current use, with the same meaning as is given to them in this work. The former word, however, is frequently used loosely to indicate certain thionic acid bacteria, and in particular *Thiobacillus thiooxidans* which in the strict sense is not one of the sulphur bacteria. Up to the present the term *Thiobacillus* has been used in the publications of American writers as a convenient catalogue name, devoid of any phyletic significance.

Genus 1.—*Thiobacillus* (Ellis).*

Colourless, motile, or non-motile, rod-shaped bacteria. Cilia presumed peritrich.

Thiobacillus Bovistus (Molisch), Ellis.

Syn. *Bacterium Bovista* (Molisch).

Literature.—Molisch (5).

Description.—A marine organism living a colonial life enclosed in bladder-like slimy colonies measuring up to 4 mm. in thickness. The outer surface of the bladder is made up of

* It has been shown (Ellis (1)) that the mode of insertion of the cilia in bacteria is a constant feature. An organism with peritrich cilia is always peritrich, and in the same way an organism with polar ciliation is constant to that mode of insertion. This is important for systematic purposes. As the majority of the rod bacteria have peritrich cilia it is advisable to assume that an organism of this class has peritrich cilia when the ciliation is unknown and, pending further investigation, to place it in the genus *Thiobacillus*.

a denser material which functions as a protective covering, and in its substance are short uncoloured rods, containing sulphur globules each about $1\frac{1}{4}\mu$ in thickness and about $2{\cdot}5\mu$ in length. The bacteria are confined to the surface layers and in the centre the material is more fluid (Fig. 23 *a* and *b*).

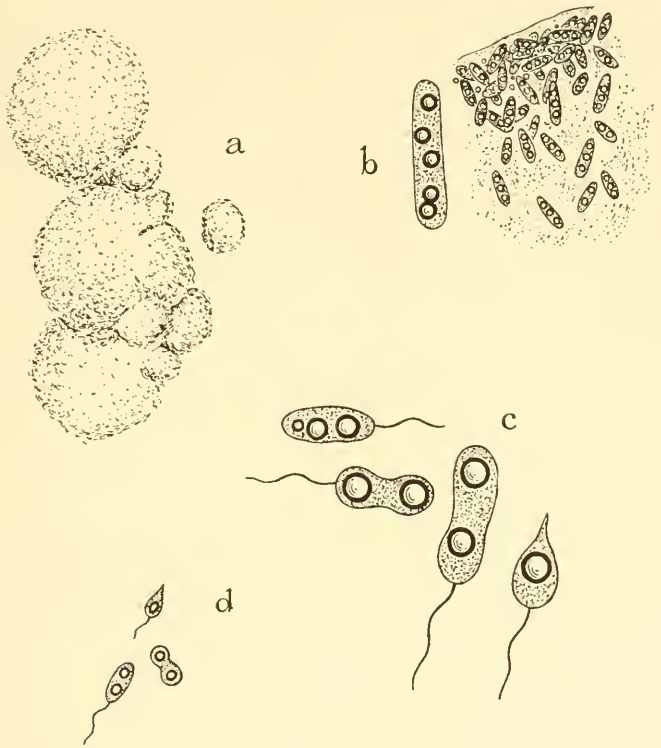


FIG. 23.

a. Thiobacillus Bovistus. × 5. *b. Thiobacillus Bovistus.*
c. Pseudomonas bipunctatus. × 1500. *d. Pseudomonas retiformans.*

The slimy globules are white in reflected, and dark or blue-black in refracted light. Under certain conditions the slime disappears and the bacteria are set free.

We must regard this organism as one in which the zoogloea condition is permanent, that condition being probably best adapted to its mode of life. The slight differentiation in the

composition of the slime seems to indicate the first step in the evolution of the group towards a more complicated organization.

Habitat.—Marine.

Thiobacillus thiogenus (Molisch), Ellis.

Syn. *Bacillus thiogenus* (Molisch).

A marine bacillus. It is described as being 0·9—1·3μ thick and 2·6μ long.

Habitat.—Marine.

Description.—The two organisms *Bacterium crystalliferum* (Gicklhorn) and *Bacterium retiformans* (Gicklhorn) do not store sulphur in the cells but deposit it outside. Accordingly they cannot be included in the sulphur bacteria. Bavendamm suggests that their place is probably in the group of thionic acid bacteria.

Genus 2.—*Thiopseudomonas* (Ellis).

Description.—Colourless, rod-shaped, motile or non-motile cells. Cilia polar.

The introduction of this genus is necessary although so far no definite species can be assigned to it. The two terms *Thiobacillus* and *Thiopseudomonas* must necessarily go together, for in most cases all motile rod-shaped forms are assigned to the genus *Bacillus*, before it is known whether the cilia are peritrich or polar. In most cases the difficulty of staining the cilia accounts for the want of knowledge as to the disposition of the cilia.

The two organisms *Pseudomonas bipunctatus* (Gicklhorn) and *Pseudomonas hyalina* (Gicklhorn) have been erroneously placed in the genus *Pseudomonas* by their discoverer. In their general character they have no resemblance to the organisms placed in this genus. They are described here because they have been labelled as sulphur bacteria on the strength of their habitat, namely, water containing hydrogen sulphide in solution. Also, they do not store sulphur in their cells.

Pseudomonas bipunctatus. Colourless transparent cylindrical cells, 8—12μ long and 3—5μ broad. The single polar

cilium is invariably behind, and about 12μ in length. The cell moves very slowly, covering about 600μ per minute, and the movement consists of a slow forward rotation about the longitudinal axis. The cell contains two, sometimes three, highly refractive drops of calcium carbonate. Multiplication is by simple fission, the cell becoming more and more constricted in the middle until separation takes place. The daughter cells remain pear-shaped for some time after separation (Fig 23*c*).

Pseudomonas hyalina. This species differs from the preceding in being only 4—6μ long and 2—2·5μ broad. Gicklhorn considered them to be distinct species from his failure to find intermediate forms. The organisms *P. bipunctatus* and *P. hyalina*, members of the thionic acid bacteria, are further treated in Chap. XII.

CHAPTER VIII.

RHODO-THIOBACTERIA (COLOURED SULPHUR BACTERIA).

Family 1. *Lankesteraceæ*. Genus 1. *Lankesteron*.
Family 2. *Chromatiaceæ*. Genus 1. *Chromatium;* Genus 2. *Thioporphyra*.

Family 1.—*Lankesteraceæ*.

Literature.—Lankester (1), 1873, and (2) 1876; Warming 1876; Zopf (1), 1882; Ellis (11), 1929.

A family of highly pleomorphic species which may be filamentous, globular, ellipsoidal-cylindrical, or spiral. The organisms enter the zoogloea condition in any of these phases. May be motile or non-motile. One genus, *Lankesteron*.

Proof of the occurrence of pleomorphism in the sulphur bacteria was submitted in Chap. I. If the facts there given are accepted, the three organisms, *Bacterium rubescens* described by Ray Lankester, *Bacterium sulfuratum* described by Warming, and *Beggiatoa roseo-persicina* described by Zopf, must be accepted as highly pleomorphic species. Hitherto each has been regarded as a mixture of species, and so has not found a place in existing classifications. The three organisms show a sufficient resemblance to justify their inclusion in the same genus.

The names *Bacterium* and *Beggiatoa* are obviously unsuitable. The former has already two meanings in bacteriological literature, and the term *Beggiatoa* is already in use to indicate a genus in which *Beggiatoa roseo-persicina* would be unsuitably placed. The term *Lankesteron* is suggested as a generic name for the inclusion of these species, and from it we derive the family name of *Lankesteraceæ*.

Genus 1.—*LANKESTERON* (ELLIS).

Literature.—Lankester (1), 1873, and (2), 1876; Warming, 1876; Zopf (1), 1882, (2), 1885, and (3), 1895; Winogradsky (1), 1887, and (2), 1888; Migula (1), 1887, and (5), 1904; Bergey, 1923; Bavendamm, 1924; Buchanan, 1925, and Ellis (11), 1929.

A widely distributed genus, found in marine and fresh waters containing plant and animal remains. Characteristics as for the family *Lankesteraceæ.*

LANKESTERON RUBESCENS (Lankester), Ellis.

Syn. *Bacterium rubescens* (Lankester). For the description of this species see page 137, Fig. 24.

LANKESTERON SULFURATUM (Warming), Ellis.

Syn. *Bacterium sulfuratum* (Warming). See page 138, Fig. 25. Warming mentions *Monas vinosa* (Ehrenberg), *Monas erubescens* (Ehrenberg), *Monas Warmingii* (Cohn), and *Rhabdomonas rosea* (Cohn) as synonyms.

LANKESTERON ROSEO-PERSICINA (Zopf), Ellis.

Syn. *Beggiatoa roseo-persicina* (Zopf). There are numerous phases in the life-history of this organism, and numerous synonyms for these phases, as for example, *Lamprocystis roseo-persicina; Protococcus roseo-persicina; Pleurococcus roseo-persicina; Clathrocystis roseo-persicina; Cohnia roseo-persicina; Thioroseo-persicina.*

A description of this organism is given on pages 140-141, Fig. 26.

Family 2.—*CHROMATACEÆ.*

Free, motile, cells, normally of a cylindrical-elliptical shape. One polar cilium. Reproduction by transverse fission.

Genus 1.—*CHROMATIUM* (PERTY).

Literature.—Ehrenberg (1), 1838; Weisse, 1845; Perty, 1852; Cohn (1), 1875; Warming, 1875; Zopf (1), 1882; Winogradsky (1), 1887, and (2), 1888; Engelmann (3), 1888; Bütschli (1), 1890, and (3), 1896; Zettnow (1), 1891, and (2), 1897;

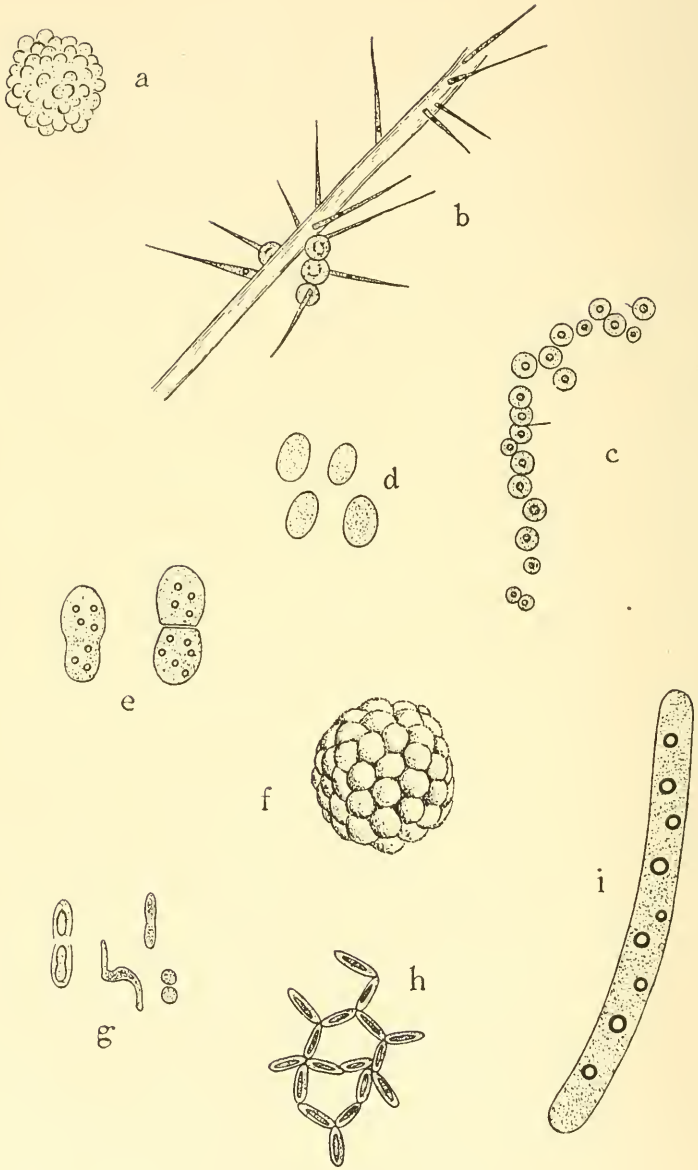


Fig. 24.

Förster, 1892; Beijerinck (1), 1893; Mitrophanow, 1893; Migula (2), 1895; Zopf (3), 1895; Ewart, 1897; Fischer (3), 1897; Bütschli (4), 1902; Nadson (2), 1903; Russel, 1909; Dangeard (1), 1909; Strzeszewski (1), and (2), 1913; Issatchenko (2), 1914; Buder (2), 1915; Lauterborn (7), 1915; Metzner, 1920; Potthoff (1), 1921, and (2), 1922; Gicklhorn (2), 1921; Bergey, 1923; Bavendamm, 1924; Scourfield, 1925-26; Buchanan, 1925; Ellis (11), 1929.

FIG. 24.—*Lankesteron rubescens* (Lankester), Ellis.
Syn. *Bacterium rubescens* (Lankester).

Some of the forms of this organism as found on decomposing caddis worms (after Lankester):—

a.—A "sphærous combination of cells forming a dark-coloured irregular zoogloea mass." (So described by Lankester.)

b.—Acicular and sphærous forms in attachment to another organism. The extremities of the acicular forms showed oscillatory movements.

c.—Chains of cocci in filamentous formation. (Zopf records a similar formation resulting from the break-up of a filament into cocci.) This "catenular aggregation," as named by Lankester, probably arose in a similar way.

d.—Biscuit forms. Described as "loose and gloeoginous."

e.—Biscuit forms.

f.—A globose aggregate of spherical units.

g.—Irregular forms.

h.—Reticulate aggregation of ellipsoidal cells. These recall the "Amœbobacter" of Winogradsky (see p. 170).

i.—"Multilocular filament." The dense cell contents in such cases were probably disposed so as to give the impression of a cell divided into several compartments.

The bright colour imparted to the water in which *Chromatium Okenii* develops, and the interesting organism itself have long made it a favourite object of study. It is the first to be noticed in a group of different microorganisms on account of its comparative largeness, its motility, and its bright colour. The genus was established by Perty in 1852, although the type organism *Chromatium Okenii* was recorded by Ehrenberg as early as 1838, and named *Monas Okenii*, under the impression that it belonged to the Monas group of *Flagellates*. Later, other species were added, differences in size being the chief

feature selected as a basis for division. At first three species were distinguished and named as follows :—

Monas Okenii, 7·5—15μ × 5μ.
Monas vinosa, 2·5μ long.
Monas Warmingii, somewhat longer than the first.

The genus was removed from the *Flagellates*, renamed *Chromatium* by Perty, and placed among the *Bacteria*. Perty

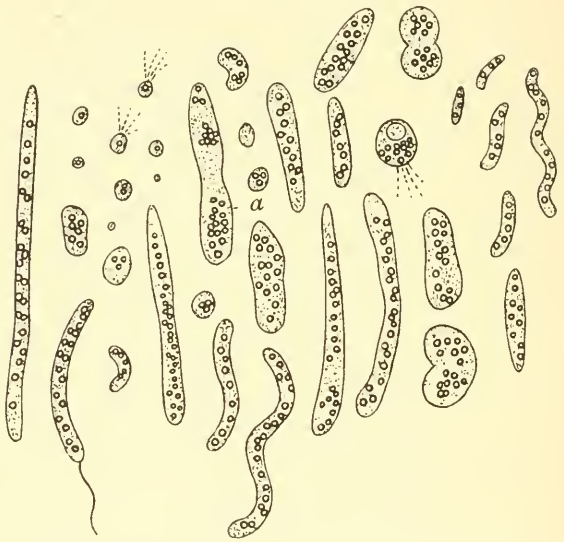


Fig. 25.—*Lankesteron sulfuratum* (Warming), Ellis.
Syn. *Bacterium sulfuratum* (Warming).

Some of the hundreds of the different forms figured by Warming as pleomorphic forms of this organism. The figure marked *a* is the *Rhabdomonas* of later writers.

added to the genus two other species, which he named *Chromatium Weissii* and *Chromatium violascens*. Later the number was added to by Winogradsky, who named *Chromatium minus* and *Chromatium minutissimum ;* and within recent years *Chr. Linsbaueri* was added by Gicklhorn, *Chr. gracile* by Strzeszewski, and *Chr. Gobie* by Issatchenko.

Description.—Coloured, free, motile cells, normally of ellipsoidal-cylindrical form, but subject to considerable variations

in shape, ranging from a very much elongated form to one that is completely spherical. Multiplication is by simple fission, the cell usually dividing at the middle. In one species a process has been observed which may be sexual.

Chromatium Okenii (Ehrenberg), Perty.

Literature.—As for genus.

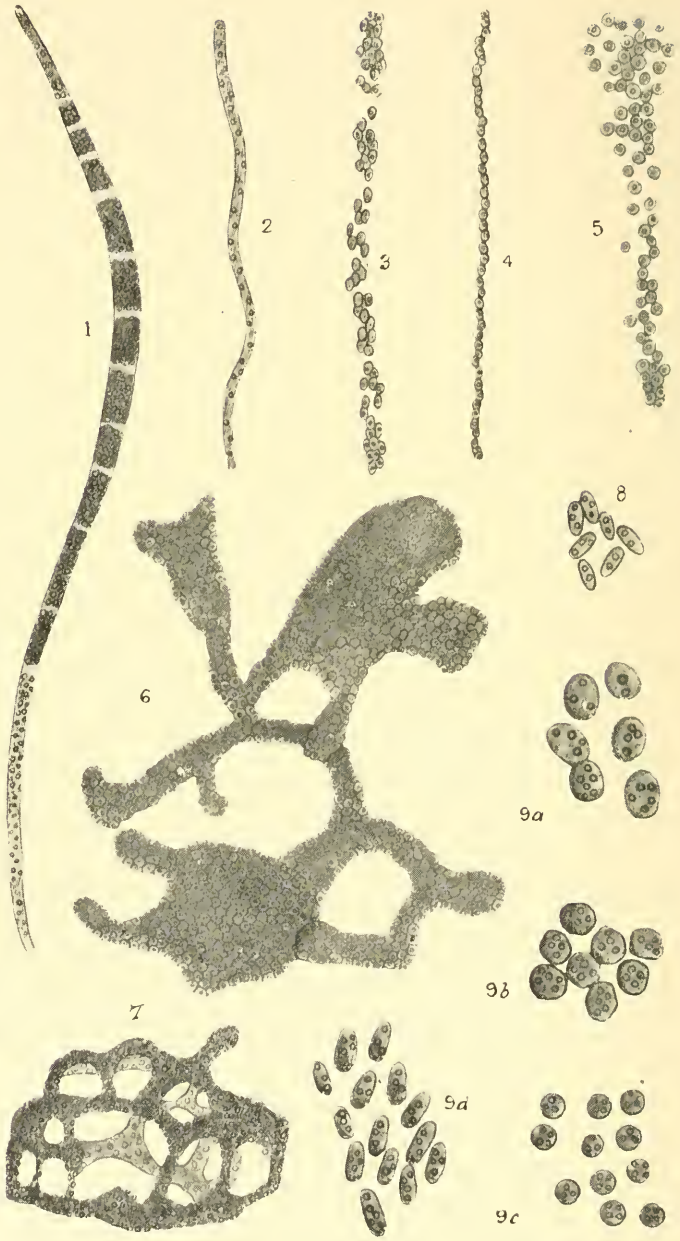
Description.—It is somewhat doubtful whether all the forms described under the name of *Chromatium Okenii* by different investigators are the same species. It is typically a free, wine-red, coloured, motile organism, of an elliptical-cylindrical shape. Motility appears to be due to the lashing of a single large wavy cilium, which is readily made visible by staining with carbol-fuchsin (Fig. 27*b*). However, it has been shown by Kolkwitz that the apparently single cilium is compound and that it may be composed of as many as forty cilia. He found that when the slime which covers the cilia was removed, the individual cilia came apart. When completely separated from the slime they appear as very fine, wavy, filaments, that are on the margin of visibility. Even in healthy cultures, some forms may be observed which are strikingly different from the normal shape. Thus bean-shaped or club-shaped individuals are not uncommon in such cultures; and there is often a noticeable variation in their size. Scarcely two observers agree on the range of its dimensions. The average is

$$7{\cdot}5—15\mu \times 5{\cdot}0—6{\cdot}3\mu.$$

Another range that has been given is

$$16\mu \times 6{\cdot}0—6{\cdot}3\mu.$$

Buder (1) has given 20μ as an extreme length for normal specimens. Warming has shown that *Chromatium Okenii* may exhibit a large range of pleomorphic forms; and Zopf (1) has come to the conclusion that so many intermediates may be found between *Chr. Okenii* and *Beggiatoa roseo-persicina* that these two apparently widely different organisms may be regarded as the same species. The point cannot be settled without further research.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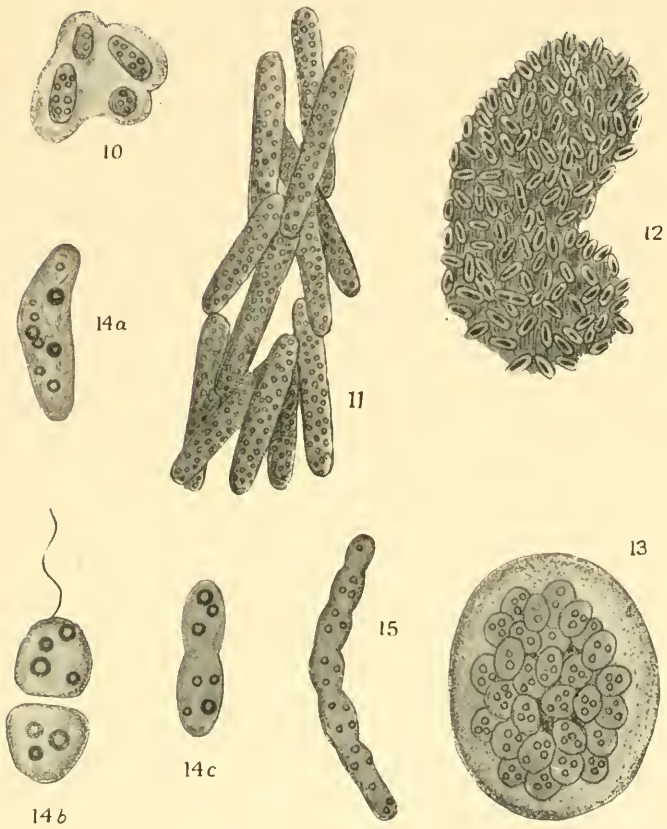
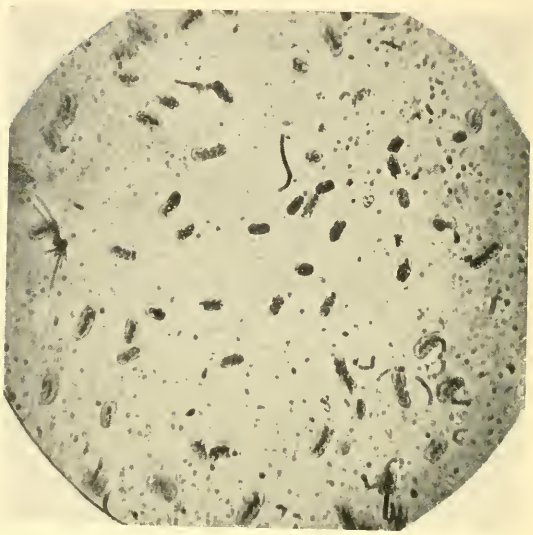


FIG. 26.—*Lankesteron roseo-persicina.*

(Diagrams after Zopf.)

1. Thread-form of this organism. × 300.
2. Small thread in undivided condition. × 300.
3-5. Thread fragments in process of transformation into cocci. × 900.
6. Large, branched and folded colony of cocci. × 500.
7. Net-form colony of cocci. × 250.
8-9. Various shapes and sizes of cocci. × 250.
10. Zoogloea of one coccus and three rod forms. × 900.
11. Zoogloea of large thick rods or short filaments. × 900.
12. Rod forms in massed formation. × 900.
13. Zoogloea of large cocci. × 900.
14-15. Various other forms assumed by this organism. × 900.



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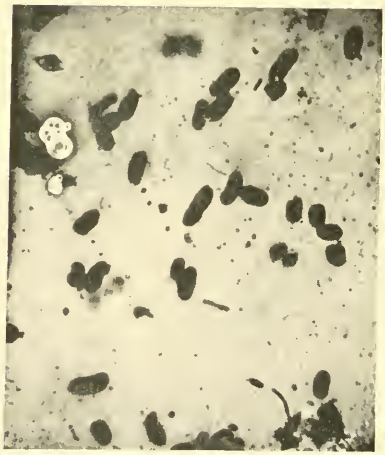

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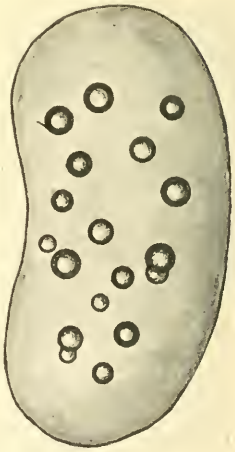


c

FIG. 27.—*Chromatium Okenii* (Ehrenberg).

a.—Photomicrograph of *Chr. Okenii*, showing the inclusions of sulphur grains. × 200.

b.—Another photomicrograph, one organism, showing the single large compound cilium. × 500.

c.—Diagrammatic representation of one of the cells, showing sulphur granules, each showing as a thick black ring with a clear centre. × 16,000.

Variant forms of Chromatium Okenii.—The tendency of this species to vary both in size and in shape has been sufficiently emphasized. The following organisms are, in the author's opinion, merely variants of the highly pleomorphic *Chromatium Okenii.*

(*a*) *Chromatium Okenii forma Weissii.*

Syn. *Chromatium Weissii* (Perty).

Literature.—Perty, 1852; Winogradsky (2), 1888; Miyoshi, 1897; Strzeszewski (2), 1913; Lauterborn (7), 1915; Bavendamm, 1924.

Elongated form about 11·5μ long and about 4·2μ thick. Connected by transitional forms with the normal type of *Chromatium Okenii.*

Fig. 28.—*Chromatium Weissii, Chromatium minus, Chromatium viriosum, Chromatium minutissimum* (arranged in order from left to right).

(*b*) *Chromatium Okenii forma minus.*

Syn. *Chromatium minus* (Winogradsky).

Literature.—Winogradsky (2), 1888; Migula (3), 1897; Strzeszewski (1), 1913; Bavendamm, 1924.

Described by Strzeszewski as an organism with numerous intermediates bridging it to *Chromatium Okenii forma Weissii.*

(*c*) *Chromatium Okenii forma minutissimum*

Syn. *Chromatium minutissimum* (Winogradsky).

Literature.—Winogradsky (2), 1888.

Cells spherical or ellipsoidal, 1—1·2μ in diameter. Individual cells are colourless, but when seen in masses are of a peach-flower colour.

Issatchenko (4) records that this organism can be made to grow in a test-tube filled with mud and water, forming a rose-coloured film on the surface, which is identical with a similar

formation that he observed at a depth of 13 metres in Lake Mohilni. He adduces this fact, together with the fact that sulphur bacteria collect round an algal filament which is liberating oxygen, in proof of his contention that the genus *Chromatium* is aerobic and not anaerobic, as reported by Bredemann.

(*d*) *Chromatium Okenii forma gracile.*

Syn. *Chromatium gracile* (Strzeszewski).

Literature.—Strzeszewski (1), 1913.

Ellipsoidal cells 2—6μ × 1—1·3μ. Single cells appear colourless, but in masses, red.

Habitat.—Sulphur springs in Krakau (Poland).

Chromatium Warmingi (Cohn), Migula.

Literature.—Cohn (10), 1875; Warming, 1875; Engelmann (10), 1888; Ewart, 1897; Bavendamm, 1924.

Description.—This is a doubtful species. The name is usually given to a *Chromatium* with the measurements 15—20μ × 8μ. The shape is thus slightly different from that of the normal *Chromatium Okenii*. Cohn named it after Warming, but Warming himself sketches it as one of the many forms assumed by *Bacterium sulfuratum*. It is also claimed that its motility is greater than that of *Chromatium Okenii*. Each individual has a single large polar cilium, which is probably compound. Bavendamm states that sulphur globules collect in the cells only during division, when they increase in numbers, and travel to the opposite ends of the dividing cells. Bavendamm succeeded in obtaining pure cultures of this organism, and as the cells in such cultures differed in size from the normal, he named this variant *Chromatium Warmingii forma minus*. The same investigator also observed the development of buds which in some cases had extended their lengths, and become attached to similar buds from neighbouring cells. The process is regarded by this writer as being possibly the first phase of a sexual method of reproduction (see Fig. 29). The probability is greater that the formation of the buds is a new

form of vegetative development that has come into being as a result of new and unaccustomed conditions of growth, and that the fusion is due to contact irritability. The new conditions of growth are certainly responsible for the marked change observed in the size of the cells. The buds are more likely to be comparable to the "clamp connections" that are found in some of the Fungi, and are, therefore, more of the nature of simple vegetative fusions or mixing of plasma from different

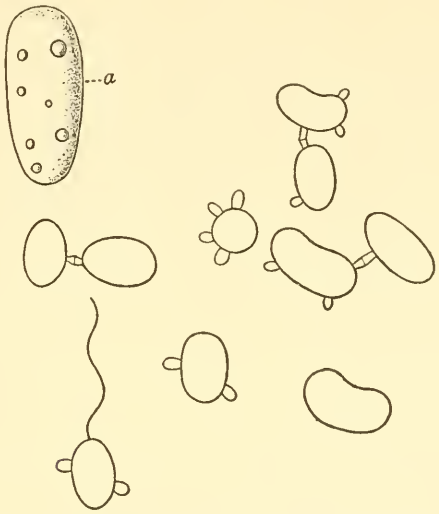


FIG. 29.—*Chromatium Warmingii forma minus* (after Bavendamm), showing the development of buds in artificial cultures of this organism. The buds of different individuals are observed to be united. For purposes of comparison of size a cell of the normal *Chr. Okenii* is shown at *circa* × 2000.

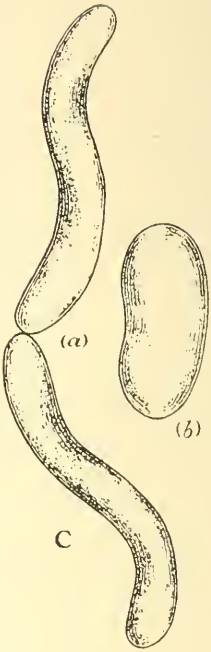
sources. The unions observed in Bavendamm's cultures appear to be similar to the H-connection in the Fungi and some of the Florideæ, which are not sexual.

CHROMATIUM LINSBAUERI (Gicklhorn).

Literature.—Gicklhorn (1), 1921; Bavendamm, 1924; Scourfield, 1925; Ellis (11), 1929.

Description.—This species is distinguished from *Chr. Okenii* by its lime content, and more particularly by its peculiar

A



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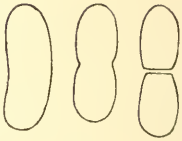

D

Fig. 30.

disposition of slime in the cell. A thin layer is formed immediately inside the membrane (Fig. 30A), and, in addition, there are one or two round patches of the same material, either together, or separate. These patches may be situated in any part of the cell (Fig. 31). This organism was found in a pool in Epping Forest, the water of which is periodically coloured a blood-red by it. Usually the red colour appears in autumn, and lasts for a few weeks when the pool again becomes clear and remains so until the following autumn. The Epping Forest overlies a bed of chalk, so the water is rich in lime. The other microorganisms in the same pond, however, did not appear to have absorbed the lime in any quantity. The richness in the content of lime makes it probable that the organism exercises a selective action in the

FIG. 30.—*Chromatium Linsbaueri* (Gicklhorn).

A. Appearance of cell in certain conditions after treatment with carbol-fuchsin. Immediately behind the membrane is a thin layer of slime which extends round the whole cell inside this membrane. The coloured globules are probably nitrogenous reserve material, and the uncoloured ones lime corpuscles. × 5000.

B. The same cell unstained and observed "end on." The centre is occupied by a large vacuole. All the globules are situated in the peripheral plasma. The globules which appear to occupy the vacuole in this diagram are in reality placed in the plasma on the far side of the cell, which is here viewed "end-on". × 6000.

C. The normal (*b*), and the pleomorphic type (*a*) of *Chromatium Linsbaueri*.

D. Shows the stages in fission of this species.

absorption of this substance, but whether it is utilized in its metabolism is not yet ascertained. The cells are ellipsoidal-cylindrical, and measure up to 15μ in length and about 6—8μ in breadth (Fig. 30). In the natural state, with one exception, no marked deviation from the normal shape was observed. This exception was a spiral form which appeared in some cultures, and was in every other respect similar to the normal organism. This is obviously a pleomorphic form. The dimensions of the "Spirillum" were constant so far as this could be determined from the small number of crude cultures in which it appeared; and there appeared in this case to be no intermediate forms. The sudden appearance of this pleomorphic form was not a frequent phenomenon. Sometimes

the cell is filled with the lime and the stainable granules, at other times the cell is filled with sulphur globules (Fig. 30c).

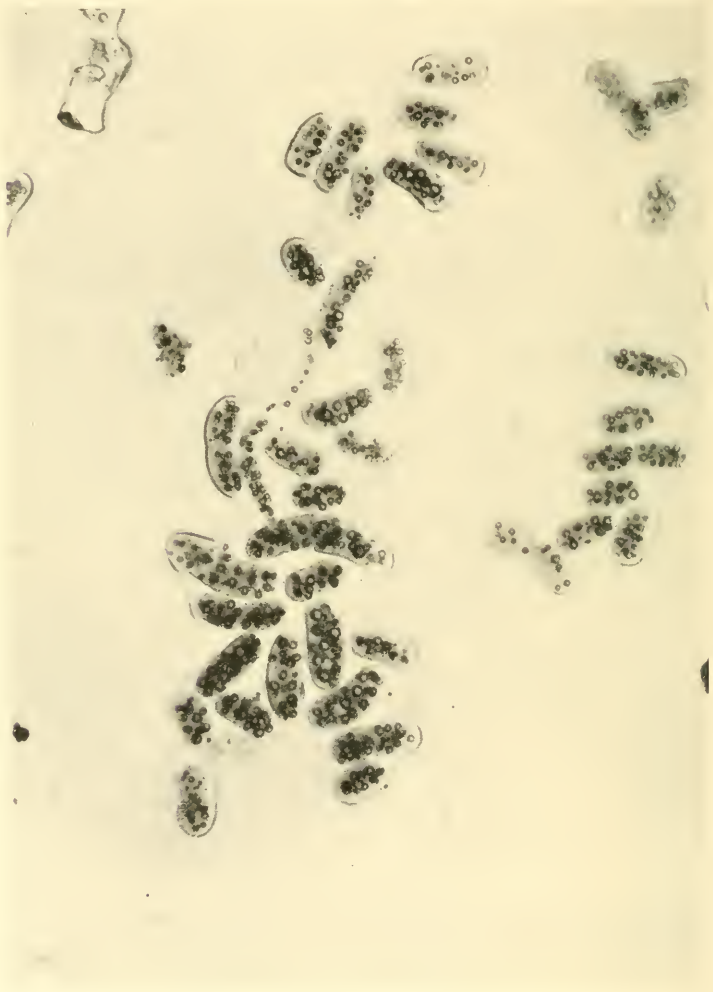

FIG. 31.—Photomicrograph of *Chromatium Linsbaueri* (Gicklhorn). × 960. (Photograph taken by Mr. H. W. Thorp, B.Sc.) The cells are almost completely filled with sulphur globules.

The intimate structure of the cell is described on pages 186 *et seq.* Gicklhorn, who was the first to describe this species,

regarded its capacity for storing lime as an outstanding feature, and considered it to be the first of a new class of bacteria, which he named *lime bacteria*, because the globules are composed of calcium carbonate. It must at present remain doubtful whether lime takes an active part in the metabolism of this organism, for its appearance seemed to be only occasional, and to accompany the formation of the stainable globules which are evidently reserve material. It is possible that it may be a waste product in the process which results in the formation of the nitrogenous reserve material (the stainable globules). The matter must await further investigation. The cell contents vary somewhat, the cell being sometimes full of sulphur granules, and at other times of lime granules and the stainable corpuscles already mentioned. Nothing is at present known of the conditions determining these differences, but they are probably due to environmental changes. (Compare Fig. 30A and Fig. 31.)

Chromatium violascens (Perty).

Literature.—Perty, 1852.

The violet colour is the distinctive feature of this organism. It was found on the wall of a glass vessel containing decomposing *Chara*. The cells are described as spherical or ellipsoidal, 2—3μ long, and very variable in size and shape.

Chromatium cucullliferum (Gicklhorn).

Description.—The cell is globular or slightly ovoid, and measures 6 × 4μ. According to Gicklhorn its very small size distinguishes the species from others of the same genus. It possesses a single cilium, and rotates very slowly about its longitudinal axis.

Description.—The sulphur globules are congregated at one end, whilst the cilium is invariably attached to the other end (Fig. 32).

The cells are colourless, and the size constant.

Habitat.—In water containing decomposing Algæ, taken from Botanic Gardens, Graz.

The absence of colour is a feature which makes questionable its inclusion in the genus *Chromatium*. Bavendamm, however, identifies it with *Chromatium Warmingii*

forma minus, chiefly on account of the characteristic massing of the sulphur globules at one of the poles. He therefore regards this organism as one potentially capable of developing colouring matter, for *Chromatium Warmingii forma minus* is the organism which was artificially cultivated by Bavendamm, and in such cultures the colouring matter was richly developed.

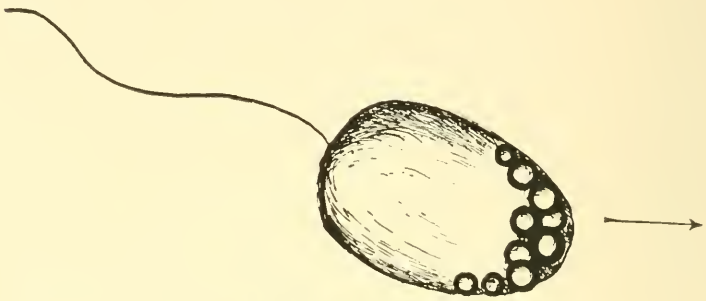

FIG. 32.—*Chromatium cuculliferum*. × 6000.

RHABDOMONAS AND *RHABDOCHROMATIUM*.

Literature.—Cohn (10), 1875; Warming, 1875; Zopf (1), 1882; Mitrophanow, 1893; Zopf (3), 1895; Bütschli (4), 1902; Nadson (2), 1903; Kolkwitz (3), 1909; Lauterborn (7), 1915; Gicklhorn (2), 1921; Bergey, 1923; Bavendamm, 1924.

The term *Rhabdomonas* is of early origin, and was employed by Cohn to designate those members of the *Chromatium* group which are rod- or spindle-shaped. *Rhabdomonas* resembles *Chromatium* in every respect except in shape. When the genus *Chromatium* was transferred from the *Flagellates* to the *Bacteria*, necessitating the discarding of the name *Monas*, the name was changed to *Rhabdochromatium*. This term is now in general use, although Bergey in his classification reverts to the older name *Rhabdomonas*. As has already been shown (see pages 138-139) this peculiar shape of organism is linked up with the normal *Chromatium* by a very large number of transitional forms, and so it cannot be regarded as a distinct species.

Within recent years Bavendamm has succeeded in making artificial cultures of a species of "*Rhabdochromatium*." If the spindle shape which is the distinguishing feature of this organism were maintained in an artificial culture, a very important fact would have been established, and the difference of form, if permanent, would have sufficed to give this type of organism generic rank. But Bavendamm describes the organisms cultivated by him as made up of dull red rods of irregular shape and of varying dimensions. The length varied from short rods to lengths of 58μ. The spindle shape is only one of numerous other forms. It seems probable, judging from Bavendamm's figures and description, that he had under observation an organism probably of the type of *Beggiatoa roseo-persicina* that reacts to artificial conditions by the production of a large number of pleomorphic forms. Or, alternatively, they may have been variant forms such as are commonly seen in the cultivation of the acetic acid bacteria, such as, for example, *Bacillus aceti*. The terms *Rhabdomonas* and *Rhabdochromatium* may have their uses for descriptive purposes, but we are not justified in using them with generic or even specific rank. In the following description the terms are retained merely as labels. They designate organisms that in all probability are phases in the life-histories of pleomorphic species, that have been described under other names; our knowledge is not yet sufficiently advanced to determine the identity of the organisms of which they are the phases.

RHABDOCHROMATIUM ROSEUM (Cohn), Winogradsky.

Literature.—Cohn (10), 1875; Warming, 1875; Zopf (1), 1882, and (3), 1895; Winogradsky (2), 1888; Mitrophanow, 1893; Migula, 1894, 1895, 1900; Bütschli (4), 1902; Nadson (2), 1903; Kolkwitz (3), 1909; Lauterborn (7), 1915; Bergey, 1923.

Description.—This is the *Rhabdomonas rosea* of Cohn and of Bergey. It is composed of spindle or rod forms, of unequal thickness. Average thickness, 3—5μ. Some attain the length of 35μ.

Found in marine and fresh waters containing sulphuretted hydrogen in solution.

Rhabdochromatium minus (Winogradsky).

Literature.—Winogradsky (2), 1888.

A smaller variety than the preceding, which it otherwise resembles. Length, 5—10μ; maximum thickness, 2—9μ. Colour, rose-red.

Rhabdochromatium gracile (Warming), Migula.

Literature.—Warming, 1875; Migula (3), 1900; Bavendamm, 1924.

This is the *Monas gracile* of Warming, which was changed to its present name by Migula.

Rod-shaped cells, in which one-end is frequently thicker than the other. Length, up to 60μ. Colour, rose-red. Found in a fresh-water pond near Copenhagen.

Rhabdochromatium linsbaueri (Gicklhorn).

Literature.—Gicklhorn (2), 1921; Bavendamm (1), 1924.

This species differs from *Chromatium Linsbaueri* in being spindle-shaped, and in being destitute of the slime patches which are characteristic of that organism. As in other respects the two forms agree, and as the marks of difference are probably of a fugitive and unstable character it is highly probable that the two organisms are both varieties of the same species. This variety is described as being 30μ long and 3—4μ broad; it is propelled by a cilium 20—30μ long. The colour is wine-red. The cell contains calcium carbonate as well as sulphur granules.

Habitat.—Found in a small pool near Graz (Austria).

Genus 2.—*Thioporphyra* (Ellis).

Literature.—Ellis (9), 1926.

Genus with one species. Cells spherical, or ovoid, motile. Movement by one large cilium which is probably compound. Colour from violet to mauve. Reproduction by transverse fission, by budding, and possibly by endospores.

Thioporphyra volutans (Ellis).

Literature.—Ellis (9), 1926.

Description.—Mass cultures are usually found as a purple mantle covering seaweed decomposing in shallow pools. Under the microscope the large purple cells, of an ovoid or spherical form, which are in active movement, are striking objects. In addition, the cells contain numerous sulphur inclusions.

The organism is normally either a large unicoccus (Fig. 34*a*) or a large diplococcus (Fig. 34*b*). Under certain conditions,

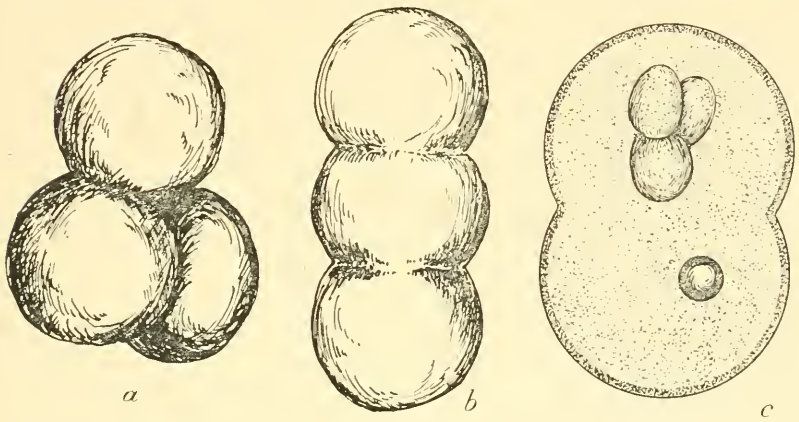

Fig. 33.—*Thioporphyra volutans* (Ellis).

a.—Tricoccus in triangular formation. × 3000.

b.—A tricoccus in linear formation. This form of tricoccus is exceptional. × 3000.

c.—A diplococcus showing three of the structures which may be endospores. In the cell a single sulphur globule, with its characteristic thick, black, peripheral ring is shown. × 5000.

connected with reproduction, tricocci may be formed (Fig. 33 *a* and *b*). The *plasma* may occupy only the peripheral portion of the cell, leaving a large vacuole in the centre (Fig. 34*d*); or it may be reticulate (Fig. 34*a*), or peripherally placed but with uneven distribution, the bulk of it being concentrated at one end if the cell is ovoid (Fig. 34*c*). The average thickness of the cells is 7μ. The purple colouring matter is diffused throughout the plasma. The cytoplasm is slightly granular, and is readily stained by iodine, methylene blue and similar reagents. It is bounded on the outside by a readily distinguishable

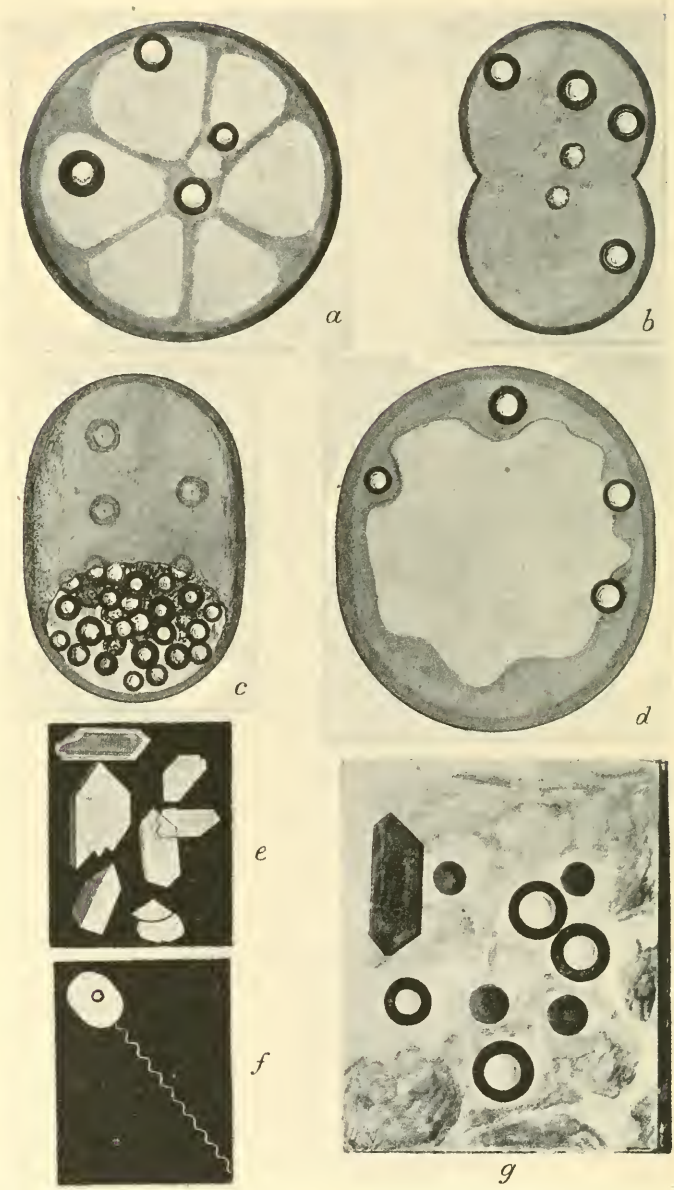

FIG. 34.

membrane. The colour varies from a deep violet to mauve. Sometimes it is perceptible in single cells, but usually only when they appear in groups. The separation of the cytoplasm from the membrane can readily be brought about by the use of concentrated reagents.

The sulphur granules may be few in number, or they may completely fill the cell; they may even be altogether absent, even in specimens that appear to be perfectly healthy.

FIG. 34.—*Thioporphyra volutans* (Ellis).

a.—Spherical cell with reticulate plasma (diagrammatic), showing uniform distribution of vacuoles. × 6000.

b.—Ovoid organism in process of division. Cell is constricted in the middle preparatory to division. In the cell are four sulphur globules and two globules from which the peripherally placed sulphur has been removed by reagents. This core is organic. × 4000.

c.—An ovoid cell in which the plasma is almost entirely disposed in the form of a cap covering about three-quarters of the periphery of the cell inside the membrane. Extensions from this cap to cover the rest of the membrane on its inner side with an extremely delicate lining may be demonstrated by special staining. The centre of the cell is occupied by a large vacuole and at one end a large number of sulphur granules are congregated. × 4000.

d.—An ovoid cell showing a third mode of distribution of plasma. The centre of the cell is occupied by a large vacuole. The plasma is more or less evenly distributed between the vacuole and the membrane. × 6000.

e.—Sulphur crystals obtained by dissolving the sulphur globules with aceto-carmine when they leave the cell and crystallize in the surrounding medium.

f.—Cell showing its single long and wavy cilium.

g.—After treatment with aceto-carmine. Some of the sulphur globules remain unaffected, in others the sulphur has disappeared, leaving a coloured centrum. One sulphur crystal is shown. × 1000.

The membrane is readily seen without the use of stains. As with all bacterial cells a layer of slime covers the membrane at all times. Under some conditions the layer is too slight to be perceptible without very careful staining. Under other conditions slime formation is extensive and a thick layer covers the membrane.

The cytology of the cell is treated with greater detail in Chap. X.

Methods of Reproduction.

(A) *Fission.*—The normal method of division is by simple fission. In normal cultures uni- and diplo-cocci preponderate,

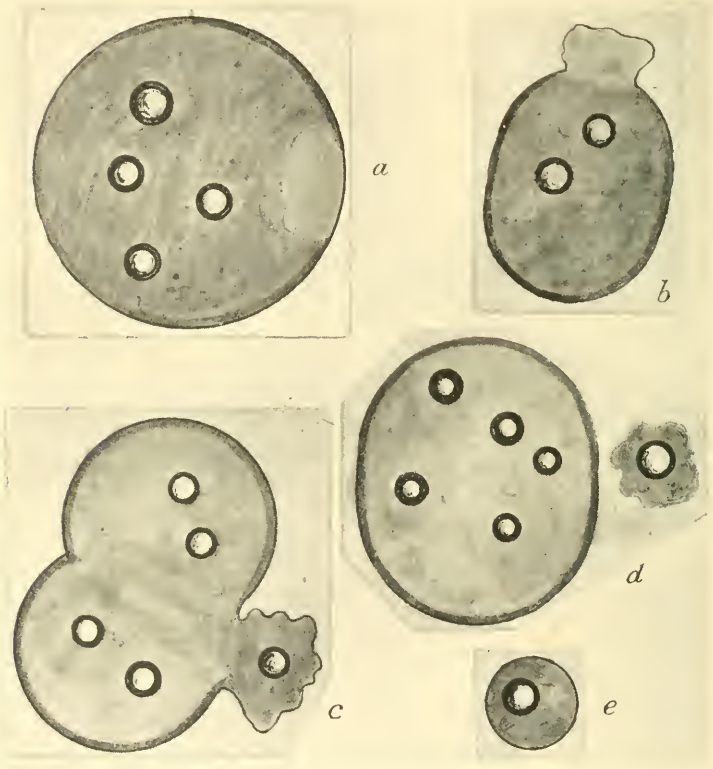

Fig. 35.—*Thioporphyra volutans* (Ellis).

Stages in bud development—

a.—First stage. Formation of a peripherally placed clear region from which the bud develops.

b.—Second stage. Protrusion of the bud.

c.—Third stage. Still further protrusion of the bud. Each contains a single sulphur globule. The bud has no external covering, and assumes a wavy outline when treated at this stage with staining reagents.

d.—Fourth stage. Separation of the bud from the parent cell. Still shows a wavy outline when stained.

e.—Fifth stage. The completed bud. Remains spherical when stained so that probably a resistant covering is formed at this stage.

the latter being evidently derived from the former. In division, the coccus elongates slightly, and becomes constricted in the middle (Fig. 34*b* and Fig. 33*c*). In many cells the process of constriction does not proceed any further, and the cell continues its existence as a diplococcus. In others, the constriction continues until complete separation of the two daughter cells takes place. Whilst division is in process, the cells are also increasing in size so that when separation take place, the daughter cells are as large as the parent coccus before division. More rarely tricocci are formed (Fig. 33*a* and *b* ; Fig. 36 at *a*). The three units in a tricoccus are usually arranged in triangular formation (Fig. 33*a* ; Fig. 36 at *a*) ; more seldom the linear arrangement holds (Fig. 33*b*). It is probable that the formation of a tricoccus from a diplococcus results from a process similar to that by which a diplococcus is formed from a unicoccus.

(B) *Budding.*—This method of reproduction, normal to the yeast plant, has not hitherto been recorded for bacteria. Under natural conditions, the organism occasionally shows a very marked increase in reproductive activity, which is marked macroscopically by an intense deepening of the purple colour. During this period more individuals are formed in a few days than are normally formed in several weeks. On the occasion of such a marked increase, it was found that the normal method of reproduction had been superseded by a method of bud production. The buds are considerably smaller than the normal form, and much more numerous. If seen apart, and in aggregated masses, they would have been denominated as a species of *Lamprocystis*. Compare Fig. 36 (1) with Fig. 36 (2).

Stages in Bud Formation.

(1) A more or less rounded space near the surface, marked off from the rest of the cell by a difference in colour, is the first indication that budding has commenced. This space is clearer than the rest, and shows a greater resistance to the penetration of stains (Fig. 35*a*). We may apply the name of *Regional Rejuvenescence* to this differentiation of the plasma,

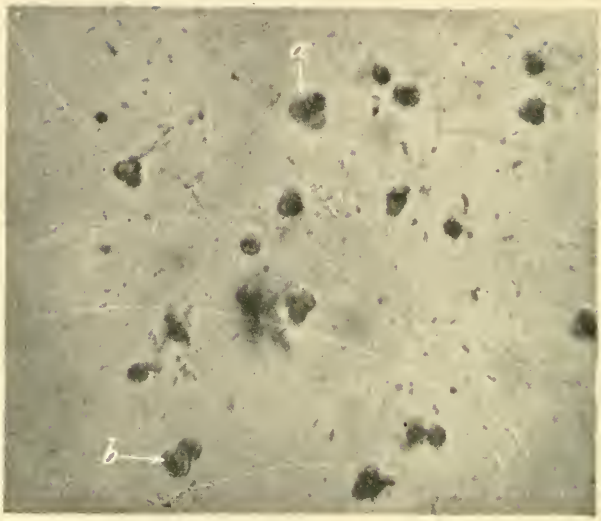

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FIG. 36.—*Thioporphyra volutans* (Ellis).

Photomicrographs taken under the same magnification (× 500) of the normal and the pleomorphic varieties of this organism. The change of form follows the change from reproduction by fission to reproduction by budding.

(1) Normal form. The majority of the individuals are unicocci. At *b* is shown a diplococcus, and at *a* a tricoccus.

(2) Pleomorphic variety. Large masses of small globules developed from the normal form by budding.

which is characteristic of *Thioporphyra volutans*, for as far as can be ascertained the bud is formed entirely from the material in this differentiated region.

(2) A protuberance appears opposite the clear space (Fig. 35*b*), and a bud emerges which is devoid of a definite membrane, and which is irregularly contoured. It was not possible to ascertain whether the membrane burst to admit of the protrusion of the bud, or whether the bud was abstricted. Usually at this stage it contains one sulphur globule.

(3) Beyond this point the development does not follow a uniform course. It may continue its growth but remain in attachment to the parent cell, in which case a bud is not formed but a unicoccus becomes a diplococcus, and a diplococcus a tricoccus. Or the bud may separate early from the parent, in which case the growth medium is occupied by a mass of small non-motile globules, each containing a sulphur globule (Fig. 35 *d* and *e*). The cells formed by budding are of uniform size and shape within certain limits.

In addition to the above, structures have been found in *Thioporphyra volutans* which may be *endospores* similar to those formed in the genus *Bacillus*, but no opportunities have occurred to study them more closely (Fig. 33*c*).

Ciliation and Movement.

The movement is one of rapid translation and rotation, by a long single cilium.

The pleomorphism of this organism has been considered in Chap. I., and the intimate structure of the cell will be discussed in Chap. X.

CHAPTER IX.

RHODO-THIOBACTERIA (COLOURED SULPHUR BACTERIA).

Family 3. *Rhodothiospirillaceæ*. Genus 1. *Rhodothiospirillum*.
Family 4. *Rhodocapsaceæ*. Genus 1. *Rhodocapsa;* Genus 2. *Rhodothece;* Genus 3. *Rhodothiosarcina*.
Family 5. *Thiocapsaceæ*. Genus 1. *Thiocapsa;* Genus 2. *Thiocystis;* Genus 3. *Thiosphærion*.
Family 6. *Amœbobacteriaceæ*. Genus 1. *Amœbobacter;* Genus 2. *Thiodictyon*.
Family 7. *Thiopediaceæ*. Genus 1. *Thiopedia*.

Family 3.—*Rhodothiospirillaceæ*.

Spirally wound, motile, coloured organisms. Ciliation polar.

Genus 1.—*Rhodothiospirillum* (Ellis).

Literature.—Ehrenberg (2), 1838; Cohn (7), 1872; Winogradsky (2), 1888; Bütschli (1), 1890; Zettnow (2), 1897; Migula (3), 1900; Buder (2), 1915; Metzner, 1920.

Winogradsky's term *Thiospirillum* was designed to include all spirilla with sulphur contents. The occurrence of these organisms in sulphur waters has been known for nearly a hundred years. Early descriptions of spiral sulphur bacteria

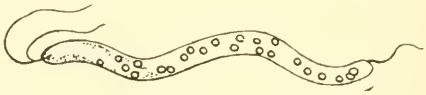

Fig. 37.—*Ophidomonas sanguinea*.

are incomplete since no distinction was made between fat globules, volutin, and sulphur globules.

In Winogradsky's genus, both coloured and uncoloured spirilla were included, so that a change of nomenclature is

necessary to conform to the system which has been adopted in this book. Hence the term *Thiospirillum* is restricted to uncoloured organisms, whilst the term *Rhodothiospirillum* is reserved for the coloured ones. Winogradsky's genus includes the spirilla described by Perty, 1852, and by Warming, 1875 ; and also Ehrenberg's two species *Ophidomonas jenensis* and *Ophidomonas sanguinea* (1838) (Fig. 37).

RHODOTHIOSPIRILLUM JENENSE (Ehrenberg), Ellis.

Literature.—Ehrenberg (2), 1838 ; Cohn (7), 1872 ; Bütschli (1), 1890, and (4), 1892 ; Zettnow (2), 1897 ; Zopf (2), 1885 ; Migula (3), 1900 ; Buder (2), 1915 ; Metzner, 1920 ; Bavendamm, 1924.

Description.—Ehrenberg gave the name *Ophidomonas jenense* to this organism, but it was changed by Winogradsky to *Thiospirillum jenense*, and for the reasons which are given above it is now again changed to *Rhodothiospirillum*. Possibly some, if not all, of the coloured spirilla may be phases in the life-history of some pleomorphic organism like *Beggiatoa roseo-persicina*, for practically all the spirilla have been named from observations of the adult specimens, without reference to their life-history.

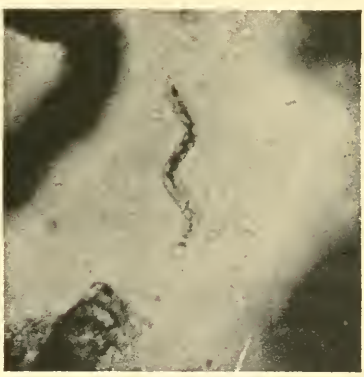

FIG. 38.—*Rhodothiospirillum jenense.*

Family 4.—*RHODOCAPSACEÆ.*

Free cells, spiral, rod-shaped, or globular, surrounded by a capsule of slime. On liberation from slime the cells may become motile. One genus with aerosomes.

Genus 1.—*Rhodocapsa* (Molisch).

Literature.—Molisch (3), 1907; Bergey, 1923; Bavendamm, 1924;

One species found in Trieste marine water, containing decomposing Zostera, to which it imparted a rose or peach-flower colour. The organism is a rod-shaped, or sausage-shaped, coloured bacillus. A slime capsule envelops each individual, although during division there are temporarily several individuals in the same capsule (Fig. 39). The cells contain peculiar structures called *aerosomes*, strongly refractive, irregularly shaped, reddish bodies, the function of which, it is claimed, is to keep the cell in suspension in the water. They are also found in the *Cyanophyceae*. The cells also contain sulphur granules. On liberation from the capsule, the cells become motile, the movement being either a rapid rotation, complete or partial, round a longitudinal axis, or rapid oscillation of one of its ends.

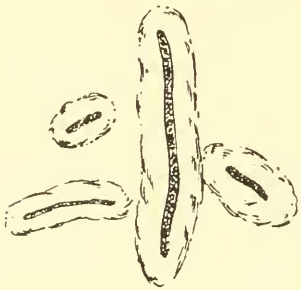

Fig. 39.—*Rhodocapsa suspensa.* × 15.

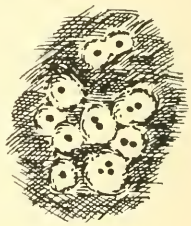

Fig. 40.—*Rhodothece pendens.*

Rhodocapsa suspensa (Molisch).

Rod- or sausage-shaped, 3·5—180μ in length and 1·8—3·5μ in thickness. Average length, 10—20μ.

Genus 2.—*Rhodothece* (Molisch), 1906.

Literature.—Molisch (3), 1907.

A genus of one species, which was found in a crude culture prepared from decaying animal remains, and sea-water, from Heligoland. The organism coloured the water as though with rose-red milk of sulphur. Like the preceding genus the cells

are enveloped in slime (Fig. 40), but they differ in being globular in shape. They are arranged either as diplococci or as short chains. Each cell measures 1·8—2·3μ in diameter, and its slimy envelope 3—14μ. Single cells do not show colour. The colouring matter is stated to be a mixture of bacteriopurpurin and bacteriochlorin (see Chap. XIII.). The cells are non-motile. One species is known, *Rhodothece pendens*.

Genus 3.—*Rhodothiosarcina* (Ellis).

Literature.—Winogradsky (2), 1888; Schroeter (1), 1889; Migula (3), 1897; Issatchenko (2), 1914; Bergey, 1923; Bavendamm, 1924.

One species in the genus. It corresponds to the *Sarcina* of the *Eubacteriaceæ*, but differs from that genus in being coloured, and in containing sulphur. The formation of packets of cocci, resembling bales of cotton, is a distinctive feature. So far as is known the organisms which are made up of packets of cocci in regular formation do not exhibit pleomorphic variations.

Rhodothiosarcina rosea (Schroeter), Ellis.

Described under the name of *Sarcina rosea* by Schroeter. Cells 2—2·5μ in diameter. Bright rose-red in colour.

Habitat.—In water containing H_2S in solution.

Family 5.—*Thiocapsaceæ* (Winogradsky).

Literature.—Winogradsky (2), 1888.

Coloured sulphur bacteria, each being a community of independent cocci enclosed in a common envelope of slime. The members of this group are perhaps phases in the life-histories of other organisms.

Remarks on the Division of Cocci.

It has been shown in Chap. VI. that organisms which normally form clusters of globular cells, and apparently divide in three planes, may under certain circumstances appear as uni- and diplo-cocci, when the number of planes in which division occurs is impossible to determine. Among the *Eubacteriaceæ* an organism made up of cocci disposed in

clusters, regular or otherwise, would be placed in the genus *Sarcina*, but under different conditions the same organism may be found as uni- and diplo-cocci, when it could equally find a place in the genera *Micrococcus* or *Streptococcus*. Hence among the *Coccaceæ* an organism made up of groups of very small denomination cannot be placed until it is determined whether its division walls are found in three planes. In some cocci this is undoubtedly the case, for the writer has observed in some, three division walls, mutually perpendicular, to be simultaneously present in the same coccus. It is felt, however, that in the vast majority of cases, organisms have been allocated to the genus *Sarcina* without ascertaining whether division took place in three planes. It has been assumed that because the organism was found in clusters it underwent this form of division. No investigation of the number of planes of division of any bacterial organism has been made to determine the constancy of the number, and yet differences in the number of planes of division have taken an important place in the classification of bacteria.

The irregular arrangement of the globular cells of the sulphur bacteria embedded in slime suggests the possibility that these organisms may be classed as *Sarcina*, but since no investigation of the number of planes of division has been made, this is a mere speculation.

Genus 1.—*Thiocapsa* (Winogradsky).

Literature.—Winogradsky (2), 1888; Migula (3), 1897; Bergey, 1923; Bavendamm, 1924.

Description.—One species only, namely *Thiocapsa roseopersicina*. The cocci which make up the organism are enclosed in slime and are about 2·8μ in diameter. The slime ultimately liquefies and the enclosed individuals escape. They are non-motile, and each settles down and forms a fresh envelope of slime. Multiplication is by fission. The colour is intense rose-red, and the cells are rich in sulphur inclusions. Winogradsky observed the development of a zoogloea for a period of six months, and found no change in its condition,

The growth of the zoogloea sometimes resulted in masses being formed which were several hundred microns in diameter.

The cells are presumed to divide in three planes by analogy with the comparable genus *Aphanocapsa* (one of the *Chroococcaceæ*) which was stated by Nageli to undergo "division alternately in all directions of space, in the successive generations."

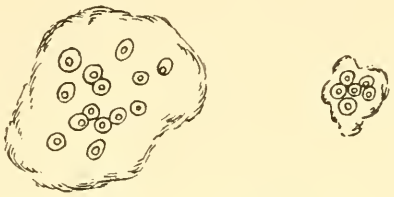

FIG. 41.—*Thiocapsa roseo-persicina.* × 2000.

Genus 2.—*THIOCYSTIS* (WINOGRADSKY).

Literature.—Winogradsky (2), 1888; Migula (3), 1897; Bergey, 1923; Bavendamm, 1924.

Families of small cocci enclosed in slime. When the slime disappears the cocci assume motility, and either separate and swim away, or enter once more into the zoogloea condition. In the former case, only a portion of the slime is liquefied, sufficiently to permit the cocci to escape.

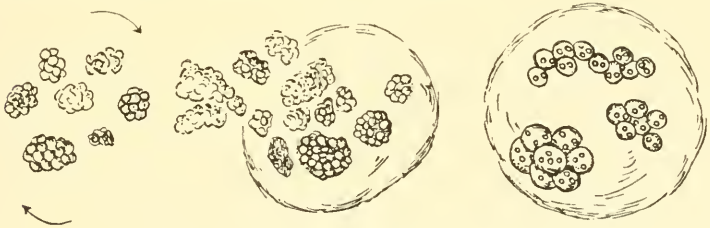

FIG. 42.—*Thiocystis violacea.* × 500.

THIOCYSTIS VIOLACEA (Winogradsky).

Cells round, 2·7—5·2μ in diameter, light rose-red or violet in colour. Slime thick (Fig. 42).

THIOCYSTIS RUFA (Winogradsky).

Cells round, not greater than 1μ. Thick slime covering; cells deep violet-red, and occasionally black with sulphur grains.

Both these species have a strong resemblance to some of the phases of *Beggiatoa roseo-persicina* which were regarded by Zopf as "one of the many kinds of the zoogloea forms of *Beggiatoa roseo-persicina*." Hence this genus is only provisionally given a place in this classification.

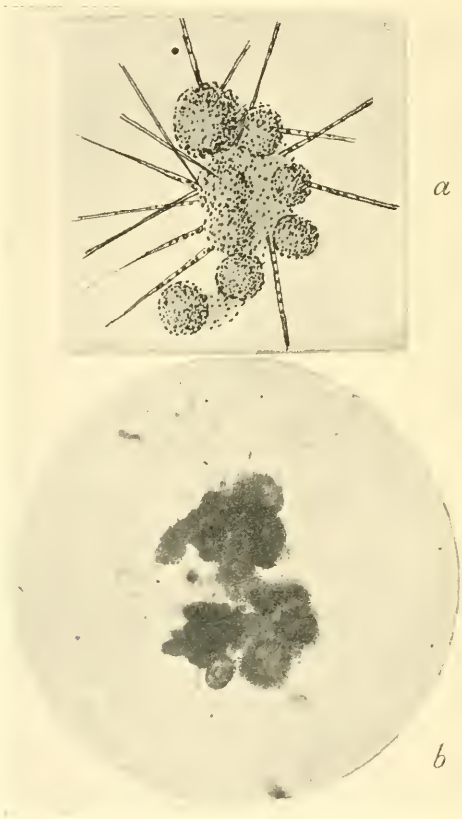

FIG. 43.—(*a*) *Thiosphærion violacea*. (*b*) Species of *Thiosphærion* (unnamed).

In Fig. 43*b* is shown an organism in the zoogloea condition, which appears to belong to the genus *Thiosphærion*. It is made up of globular masses arranged in an irregular fashion, the whole being slightly tinged with red. Each globular mass is about 15μ in diameter, and is composed of a large

number of small cocci, each containing one sulphur granule. The coccus is roughly spherical (about $1\frac{1}{2}\mu$ in diameter) and is devoid of a membrane. An opportunity has not arisen to follow the life-history of this interesting species.

Genus 3.—*Thiosphærion* (Miyoshi).

Literature.—Miyoshi, 1897; Migula (3), 1897; Bergey, 1923; Bavendamm, 1924.

Description.—The outstanding feature of this genus of only one species is the exactly spherical shape of the colonies, and their violet colour. Migula and Bavendamm included the genus in the *Lamprocysteæ*. Bergey removed it from this group, and once more gave it generic status. The exactly spherical solid masses of slime of a violet colour, arranged in little groups in attachment to objects in the water, appear to offer a sufficient distinction to merit generic rank.

Thiosphærion violacea (Miyoshi).

Cells are spherical-ellipsoidal, 2·5—1·8μ, and violet in colour. Sulphur inclusions are somewhat angular (Fig. 43*a*).

Habitat.—Sulphur springs in Japan. Found on threads of *Thiothrix*.

Note on the Organism *Thiosphæra gelatinosa* (Miyoshi).

Literature.—Miyoshi, 1897; Migula (3), 1900; Bergey, 1923; Bavendamm, 1924.

All that is known of this organism is that it is made up of ovoid cells ($7\mu \times 5\mu$) enveloped in a loose gelatinous envelope. Migula and Bavendamm placed it in the *Lamprocysteæ*, whilst Bergey gave it generic rank. As so little is known, and what is known is a condition that may be a phase in the life cycle of one of several organisms, no useful purpose is served by its retention (Fig. 44).

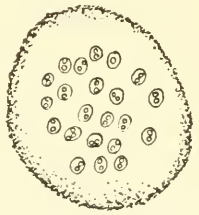

Fig. 44.—*Thiosphæra gelatinosa.*

Family 6.—*Amœbobacteriaceæ.*

Literature.—Winogradsky (2), 1888; Migula (3), 1900; Bergey (1), 1923; Bavendamm (1), 1924.

Cells united into clusters usually enclosed in slime. When movement takes place the cells move in unison.

Genus 1.—*Amœbobacter* (Winogradsky).

Description.—A peculiar group of three species. The cells are enclosed in a slime sheath, and slowly move inwards or spread outwards in unison inside the slime. As a result there is a slow alteration in the shape and mass of the community of cells. This united action of the cells was explained by Winogradsky as being due to contractile plasma threads which bound the cells together, but neither he nor Lauterborn was able to see them. It is, however, difficult to explain these co-ordinated movements except by postulating the existence of such threads. There is also another character of interest. The slime envelope is made up of two layers, an outer which is strongly refractive, and an inner which is feebly refractive. The cocci on the opening of the slime cyst do not separate, but move out together, leaving an empty shell. They then attach themselves to some object in the water and whilst in attachment exhibit those slow changes of form which are characteristic of *Amœba*. Also in each cell a perceptibly large vacuole can be discerned.

Whilst in attachment, and before slime formation has proceeded very far, the cells close in until they are all huddled together, and then they again spread out in extended formation. It is not known whether the activating force of such movements is spontaneous, or whether the movement takes place in response to external influences. Winogradsky considers that the presence of hydrogen sulphide in the water is responsible for these movements, but has not adduced proof in support of his statement. If the cells are connected by plasma threads, *Amœbobacter* must be regarded as a loosely compacted coenobium.

Amœbobacter roseus (Winogradsky).

Literature.—Winogradsky (2), 1888; Macgregor Skene, 1914; Lauterborn (7), 1915.

Each cell is globular but capable of altering its shape. Dimensions 2·8—3·4μ in diameter. The cell elongates before division to approximately 6μ. Colour a delicate rose (Fig. 45).

Habitat.—Found in waters containing sulphuretted hydro-

a b

FIG. 45.—*Amœbobacter roseus.* (*a*) Encysted condition, (*b*) Emergence of cells from cyst and formation of swarm cells. × 1500.

gen in solution. Skene obtained this organism from material supplied from Kiel, and found that he could obtain impure cultures by adding to the material sulphuretted hydrogen, ammonia (as a source of nitrogen), and chalk (as a neutralizing agent).

AMŒBOBACTER BACILLOSUS (Winogradsky).

Literature.—Winogradsky (2), 1888; Lauterborn (7), 1915.

Cells rod-shaped, 2—4μ long and 1·7μ thick. Colour red.

Habitat.—In waters containing sulphuretted hydrogen in solution.

AMŒBOBACTER GRANULA (Winogradsky).

Literature.—Winogradsky (2), 1888.

Cells very small, about 0·5μ in diameter, and almost colourless. Each contains a single sulphur globule so highly refractive that it blurs the contour of the coccus.

Habitat.—In waters containing sulphuretted hydrogen in solution.

Genus 2.—*THIODICTYON* (WINOGRADSKY).

Literature.—Lankester (1), 1873, and (2), 1876; Winogradsky (2), 1888; Migula (3), 1900; Kolkwitz (3), 1909; Bergey, 1923; Bavendamm, 1924.

Description.—An organism was sketched by Lankester in 1873 as a pleomorphic phase of *Bacterium rubescens* which

took the form of rod-shaped or spindle-shaped units, united to form a net somewhat of the same kind as *Hydrodictyon*. It differs from the preceding genus in the absence of a well-defined substantial slime covering. The same or a similar organism was found by Winogradsky, and given generic rank under the name of *Thiodictyon*. If we accept the fact of a wide pleomorphism in the sulphur bacteria we should rank *Thiodictyon* as another instance of the phenomenon. Whilst, however, it is possible that the majority of the forms observed by Lankester on the caddis worms examined by him were pleomorphic forms of one organism, it is also possible that there may have been two or even three species present and that the organism known as *Thiodictyon* was one of them.

Thiodictyon elegans (Winogradsky).

Literature.—As for the genus.

Description.—The rods form at first a compact mass, and are united to one another by their ends. Later they extend to

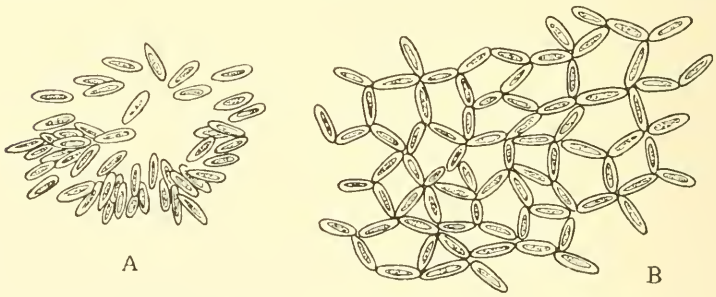

Fig. 46.—*Thiodictyon elegans*. Rod-shaped cells in process, spread to assume arrangement shown in completed state in B. × 1500.

form a net-like arrangement (Fig. 46). Under unfavourable conditions, the net arrangement is abandoned, and the units move inwards so that compact formation is once more assumed. Multiplication of the rods takes place by division, or a small number of units may be liberated after division has taken place. These grow into larger colonies. The cells are 5μ by 1·7μ long before division, but become longer during the course of the division. They are coloured a very faint red. In the peripheral plasma are a number of small sulphur granules.

Habitat.—In sulphur springs, and other waters containing sulphuretted hydrogen in solution.

Thiothece and *Thiopolycoccus.*

The genus *Thiothece* was founded by Winogradsky (2) to include a single organism, which he named *Thiothece gelatinosa* (Fig. 47). The cells of this organism are small and either globular or ellipsoidal-cylindrical. They measure 4·2μ in diameter, and it is stated without experimental evidence that they divide only in one plane. For this reason the genus is placed in the *Amœbobacteriaceæ*.

Fig. 47.—*Thiothece gelatinosa.* × 1000.

Winogradsky observed the emergence of the cells from the slime, and their subsequent transition into the swarm condition. Each cell has a single cilium. The colour of the cells is an intensely grey violet, or pale rose, or even yellowish or dirty green. The sulphur globules are located in the peripheral layer. Winogradsky also noted the similarity of the organism to one of the pleomorphic forms of *Bacterium rubescens*, but rejected the idea that the two were identical. *Beggiatoa roseo-persicina* also exhibits a pleomorphic phase that appears to be identical with the organism described by this investigator. The figure which he gives of *Thiothece* is one which it would not be difficult to match in the drawings of several of the better-known bacteria. In addition, the name was given from observation of only the adult condition of the organism, and its life-history was not investigated.

The same objections apply to the genus *Thiopolycoccus* of the same writer. This was stated to differ from *Thiothece* only in the absence of cilia after release from the slime. The differences between the two organisms which have given rise to these two genera are not sufficiently marked to warrant one being regarded as a variant of the other, far less sufficiently marked to necessitate founding two genera. It is considered that the objections just stated are such as to

warrant the removal of both these organisms from a general scheme of classification.

The *Thiopolycoccus ruber* of Winogradsky is composed of small cells closely pressed together and non-motile. They are spherical, 1·2μ in diameter, and intensely red in colour.

Family 7.—*Thiopediaceæ* (Winogradsky).

Literature.—Oerstedt (1), 1840 and 1841; Rabenhorst (2), 1865; Warming, 1875; Winogradsky (1), 1887; Utermöhl (1), Migula (3), 1900; Kolkwitz (3), 1909; Bavendamm, 1924.

Description.—The single species of which this family is composed has been known for ninety years. Oerstedt named it *Erythroconis littoralis*, but its best-known earlier name was *Merismopedia littoralis*. Warming was of the opinion that it was either a variety of *Bacterium sulfuratum*, or a small colony of *Clathrocystis*. In Migula's classification *Merismopedia littoralis* was placed among the sulphur bacteria. It was regarded as identical with the *Thiopedia rosea* described by Winogradsky, and so the term *Merismopedia* was dropped and the term *Thiopedia* took its place. The genus *Merismopedia* of to-day belongs to the *Cyanophyceæ*. The American Society of Bacteriologists has rejected *Thiopedia* as an independent genus. Its rejection is not justified, because the regularity of formation of the plates of cells is a striking feature, which is absent from all the other genera of the sulphur bacteria, and the regularity indicates a first step towards the colonial habit. This organism is thus essentially different from the other slime-enclosed bacteria in which the association of the component cells is accidental and in which the cells behave as independent units.

Genus 1.—*Thiopedia* (Winogradsky).

Literature.—As for family.

Thiopedia rosea.

Globular cells, 1·1—2μ in diameter, and arranged in the slime in regular plates. Later, the regular arrangement

is discarded and reproductive activity is intense. Colour, red (Fig. 48).

Habitat.—Widely distributed in waters containing hydrogen sulphide.

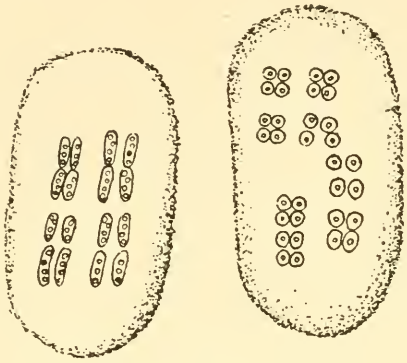

FIG. 48.—*Thiopedia rosea.* × 750.

NOTE ON "GENUS" *LAMPROCYSTIS.*

The following " species " are given for purposes of reference. All except the first were found by Miyoshi, and brought under *Lamprocystis* by Migula.

LAMPROCYSTIS ROSEO-PERSICINA.

Literature.—Kützing (1), 1833, (2), 1843, and (3), 1849; Cohn (4), 1864; Fleischer (1), 1860; Rabenhorst (2), 1868; Cohn (10), 1875; Warming, 1875 and 1876; Zopf (1), 1887; Winter (1), 1884; Winogradsky (1), 1887, and (2), 1888; Engelmann (3), 1888; Migula (2), 1895; Bergey, 1923; Bavendamm, 1924.

Spherical-ellipsoidal cells, 2·1—2·5μ in diameter. Colour intense violet (Fig. 49).

LAMPROCYSTIS VIOLACEA (Miyoshi), Migula.

Literature.—Miyoshi (1), 1897; Migula (3), 1900; Bergey, 1923; Bavendamm, 1924.

Named *Thiosphærion violaceum* by Miyoshi. Spherical-ellipsoidal cells, 2·5μ × 1·8μ, and capable of motility. Found in solid families surrounded by slime. Colour violet. Angular sulphur granules. See page 167.

Habitat.—Sulphur springs in Japan.

Lamprocystis gelatinosa (Miyoshi), Migula.

Literature.—As for preceding.

Named *Thioderma gelatinosa* by Miyoshi. Spherical-ellipsoidal cells, $7\mu \times 5\mu$. Light violet in colour with covering of slime loosely surrounding the family.

Habitat.—Sulphur springs in Japan.

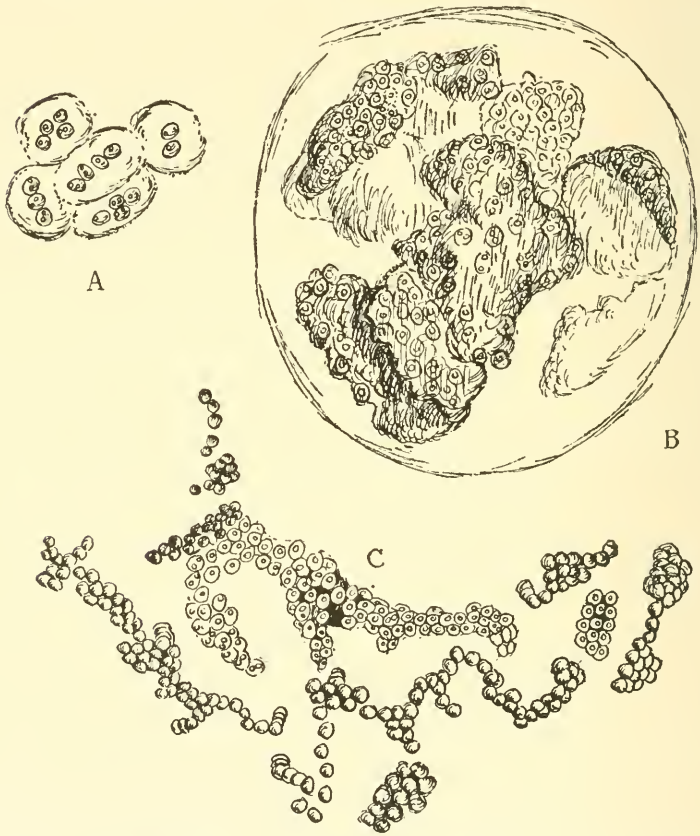

Fig. 49.—*Lamprocystis.* A. Zoogl. of *L. roseo-persic*; B. same 3 weeks later; C. same after 1 month. × 1000.

Lamprocystis rubra (Miyoshi), Migula.

Literature.—As for *L. violacea.*

Named *Thioderma rubrum* by Miyoshi. Ellipsoidal cells, $4\mu \times 2\mu$. Light red in colour and surrounded by

peach-flower red, detachable, slimy membrane. Cocci are motile.

Habitat.—Sulphur springs of Japan.

Lamprocystis rosea (Miyoshi), Migula.

Literature.—As for *L. violacea.*

Named *Thioderma roseum* by Miyoshi. Ellipsoidal cells, $2{\cdot}5 \times 1{\cdot}5\mu$. Light red in colour and covered by a thin detachable membrane of a dull purple red colour. Angular sulphur granules.

Habitat.—Sulphur springs of Japan.

The reasons for discarding this genus are given on page 89.

CHAPTER X.

THE INTIMATE STRUCTURE OF THE CELL IN THE SULPHUR BACTERIA.

Introduction.

In the *Eubacteria* the cell structure of the *Bacillus* and the *Coccus* differs essentially from that of the *Spirillum*. In the first two there is a definite peripheral cell membrane* which can be separated by plasmolysis from the rest of the plasma. The method of division is one characteristic of plant cells. When division takes place, a transverse membrane is formed across the plasma, and separation of the dividing cells is effected by an equatorial fission of this transverse membrane. In the *Spirilla*, division is effected in a far simpler fashion. The plasma retracts from a zone which extends across the middle of the cell. This appears as a hyaline space, and is occupied by slime. The daughter cells are already organically separated, but are held together by the slime. Complete separation is effected by the two daughter cells pulling in opposite directions, thereby causing the binding slime to stretch out to the point of separation. This method of division is typical rather of the simpler animal cells than of the simpler plant cells. Indeed the genus *Spirillum* shows more affinities to the animal than to the vegetable kingdom. The distinction may appear to many to justify the separation of the genus *Spirillum* from the class of Bacteria, but it must be borne in mind that all bacteria are so low in the scale of life that the distinction between an animal and a plant is not so hard and fast as in higher organisms ; and so it is not so inconsistent to place into

* The membrane in the genus *Bacillus* is not lifeless. It is the outermost, somewhat transformed but living layer of the plasma.

the same group organisms, some of which show plant-like affinities, whilst others are more nearly akin to animals.

Beggiatoa alba (Trevisan).

The filaments vary in length, and may be motile or non-motile. The *membrane* is well defined and stains more deeply than the internal plasma. It is plastic, and offers no resistance, either to the bending of the filament, or to the escape of solid cell inclusions when these are subjected to outwardly directed pressure. Plasmolysis has not been observed.

The *plasma* is reticulated, the meshes being irregular in form and variable in size. Very minute granules can be distinguished in its substance which, since they appear to be used up during growth, are probably reserve materials comparable to the basophile granules of the yeast cell. The plasma consists of strands which extend throughout the cell (Fig. 6*g*).

Sheath formation.—The outer layers may undergo transformation into a mucilaginous material which first hardens, and then separates from the rest of the organism. It forms a hollow cylindrical covering to the rest of the cell, and is known as the *sheath*. The formation of a similar sheath has been observed in the life-history of the two Iron bacteria, *Cladothrix dichotoma* and *Crenothrix polyspora*. In these it is formed early in development, persists through life, and subsequently hardens, thereby forming a sheath around the cells. In *Beggiatoa alba*, on the other hand, although sheath formation can be demonstrated in motile threads, its amount is so very small and insignificant that it can be recognized only with difficulty. It reaches full development when conditions of life become unfavourable; and its formation precedes the dissolution of the cells (see p. 94). When the protoplast under unfavourable conditions breaks up into a string of segments, sheath formation occurs between the segments, and when the latter ultimately disappear a ragged hollow cylindrical sheath is left with occasional transverse bridges of sheath material running across the hollow interior (Fig. 6*h*). A similar formation of mucilage, which similarly

undergoes a hardening process, is characteristic of yeast cells under certain unfavourable conditions. Former investigators of *Beggiatoa alba* have not distinguished with sufficient clearness between sheath-walls and cell membrane. To sum up, the cell membrane is a living, peripheral, plasmatic covering, whereas the sheath is a non-living transformation of the latter.

Sulphur globules of an oily consistency and high refractive power are found throughout the cytoplasm. The number of globules varies, and they may even be entirely absent. The variation in their number is evidently connected with metabolic changes, and their absence from the filaments usually indicates unfavourable conditions. They vary considerably in size, some being mere points whilst at the other extreme globules of 2μ in diameter may be found. They present a very characteristic appearance; a thick black ring sharply defined appears to enclose a clear interior (Figs. 6*a*, 6*g*). Each gives an appearance under the microscope comparable to a thick ring of black ink drawn on white paper. It is highly probable that the development of a sulphur droplet is preceded by the formation of a vacuole in the cytoplasm into which secretion takes place. Arthur Meyer and the author (Ellis (1)) have shown that the development of the bacterial spore takes place by secretion into a previously prepared vacuole. The development of the sulphur globule appears to follow similar lines. In the investigation into the intimate structure of the cell of *Thioporphyra volutans*, it is shown that the sulphur globule possesses a centrum of organic matter.

Tests for Sulphur.—Sulphur globules in plant cells have a characteristic appearance. See Figs. 27 (*a* and *c*), 31, 32. The centrum is hyaline. When the cells are treated with aceto-carmine, the sulphur is dissolved and crystallizes outside the cells in octohedra (see Figs. 17*d*, 34*e*) typical of this element. When treated with a concentrated solution of sodium-nitro-prusside, $Na_2[Fe(NO)(CN)_5] . 2H_2O$, the rings of sulphur assume a blood-red colour.

There is no evidence of a nucleus, and no colouring matter is developed.

Motility.—The cause of movement is at present unknown.

It is probable that cilia are not formed, and that motility is due to the power of contractibility which is inherent in protoplasm, and which here finds expression.

Thioporphyra volutans (Ellis).

Each organism is composed of either a single cell, or less frequently, of a diplococcus, or very rarely a tricoccus. The single cell is either spherical or ovate, and its diameter is approximately 7μ.

The *membrane* is well defined, and appears to be the outer limiting layer of the cytoplasm; it takes the colour more deeply, apparently because its consistency is denser than that of the cytoplasm. Its outer layers turn readily to a slimy material which forms a loose irregular mantle enveloping the coccus. The mantle is best observed when the cell is treated with the Giemsa stain. It is too thin and too delicate in texture to be regarded as a regular sheath, and it may be remarked that most, if not all, bacterial cells of the *Eubacteria* possess a similar covering. As is the case with *Beggiatoa alba*, solid objects such as sulphur globules readily pass out of the cells.

The *cytoplasm* is reticulate and readily stained. The disposition of the protoplasmic matter is described on page 153. It is finely granular, and diffused through its substance is a purple colouring matter which varies in tint from a deep violet to a mauve colour. It is visible in individual cells only if the pigment is intense. There is no nucleus.

Motility is effected by means of a single long and strongly developed cilium (Fig. 34*f*). Probably, as in *Chromatium*, this cilium is composed of several cilia united by a common envelope of slime.

The *sulphur globules* which are formed in the cytoplasm may be scattered throughout the cell, or grouped at one spot. If the cell is ovoid, the grouping is at one of the poles (Fig. 34*c*). In appearance, size, and reactions they resemble the sulphur globules of *Beggiatoa alba* (see Fig. 34 *a*—*d*, Fig. 35 *a*—*e*). It is only the outer portion of the globule that is composed of sulphur. Each contains a central core of organic matter which

is stained by aceto-carmine. Compare with sulphur globules of *Thiophysa volutans*. This reagent dissolves the sulphur and stains the core after twenty-four hours. The core can also be stained with carbol fuchsin and other reagents. It is probably a nitrogenous reserve material of the organism. The sulphur may be dissolved by chloroform, hot potassium nitrate, or 50 per cent. acetic acid; in the last named an exposure of twenty-four hours is necessary. In strong picric acid solution the globules tend to fuse to form larger irregular drops, but complete solution does not take place. They are unaffected by strong hydrochloric acid, and are insoluble in water. When dissolved, and free from the cell, they frequently combine to form typical sulphur crystals (Fig. 34*e*).

Thiophysa volutans (Hinze).

This is a colourless, motile organism, spherical in shape, which was found in the Gulf of Naples, near a submarine sulphur spring. The cocci measure 7—18μ in diameter (Fig. 17).

The *membrane* is double contoured and 0·7μ thick. It gives the reaction for pectin. Three distinct layers can be distinguished with Delafield's hæmatoxylin.

The *protoplast* consists of a very delicate hyaline layer on the inner side of the membrane, and from it radiate fine threads of plasma, the whole forming a delicate network. The central portion is occupied by a large vacuole. The cytoplasm can be separated from the membrane by plasmolysis.

The *sulphur globules* are insoluble in mineral acids and alkalis, they are partly soluble in acetic acid, and very soluble in alcohol and chloroform. In concentrated glycerine they crystallize out, and form monoclinic crystals outside the cell, several droplets uniting to form one crystal (Fig. 17*d*).

Sulphur Builders.—These are bodies ranging from 4μ to a size when they are scarcely visible. They are ovoid or round, are of a dull green colour, and occupy the vacuoles in great numbers. They stain with methylene blue, Congo red, and other reagents; are insoluble in 1 per cent. potassium hydrate,

and in 10 per cent. sodium chloride. Hinze regarded them as sulphur builders because they resemble the remains which are found after the sulphur droplets have been dissolved out. His conclusion that they are "builders" of sulphur is conjectural (compare the sulphur globules of *Thioporphyra volutans*).

Other statements made by Hinze require confirmation, especially where he distinguishes in the cytoplasm a distinct wall which his figures do not show, and where he refers to small bodies embedded in the wall as being chromatin. If this is so it demonstrates the essentially living nature of the membrane. The author (p. 189) has shown that the cilium of *Chromatium Linsbaueri* is directly connected with the membrane and does not pass through, a fact which is confirmatory of the same conclusion.

Beggiatoa mirabilis (Cohn).

This organism was described by Cohn (5), by Warming from material found on the Danish coast, and by Engler who found it in the Kiel Canal. It is doubtful whether all three have described the same organism. All describe a species that is relatively very large, for its thickness may reach up to 45μ, and so it is exceptionally favourable for the study of the intimate structure of the cell. Cohn sketches definite transverse membranes of a typical plant character (Fig. 8*c*); whilst Warming states "j'ai pu alors distinguer des cloisons avec des interstices de 2·5 a 3·5μ," and depicts threads (Fig. 8 *a* and *b* and Fig. 50) in which it appears as though the division of the thread into segments was not effected by transverse walls, but rather by plates of mucilaginous matter such as are observed when segmentation occurs in threads of

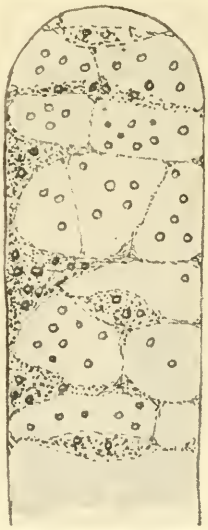

FIG. 50. — *Beggiatoa mirabilis* (Warming). × 750.

Beggiatoa alba. Also, if Warming's drawings are correct, the threads divide by simple fission, a process in which the formation of transverse walls plays no part.

Hinze (1—2) examined an organism under this name which evidently tallies with the *Beggiatoa mirabilis* of Cohn, but not with that of Warming. The membrane is double contoured, and is not composed of cellulose. The transverse walls are thinner than the outside walls and stain with ruthenium red, safranin, and other reagents that stain pectin bodies. Inside the wall is a peripheral layer of plasma (Rindenschicht) in which are numerous fine red granules which stain with hæmatoxylin and alleged by Hinze to be chromatin. Inside this is a large vacuole occupying the chief space of the cell and containing a large number of solid granules which often show molecular movements. The plasma is granular, homogeneous, and contains numerous sulphur granules.

Chromatium Okenii.

The observations on the intimate structure of this well-known and much-studied organism are few and unsatisfactory.

Dangeard states that a colourless membrane encloses an alveolar cytoplasm, and that the colour is diffused throughout the cytoplasm. He made out a "Centralkörper" which corresponded to the Centralkörper of the *Cyanophyceæ*, and regarded this as the nucleus of the cell. Attempts to distinguish in bacterial cells in general between a peripheral and a central body (Rindenschicht and Centralkörper) date from Bütschli's investigations, and his conclusions are nowadays not accepted. If we may judge from the structure of the allied species *Chromatium Linsbaueri* it seems as though Dangeard's conclusions were influenced more by Bütschli's conception of the cell than from the evidence of structure presented by the cell itself.

Achromatium oxaliferum (Schewiakoff).

Under the various names of *Achromatium oxaliferum* (Schewiakoff), *Modderula Hartwigi* (Frenzel), and *Hillhousia mirabilis* (West and Griffiths), the intimate structure of the

cell of this species has received close attention, especially at the hands of West and Griffiths, and of Virieux.

The *cell wall* is well defined, double contoured, and is not composed of cellulose. It is easily separated from the protoplast by plasmolysis. It measures 2—3μ in thickness and can be made to swell by the use of various reagents. When thus treated the swollen outer layers show, with high magnification, a mass covered with minute granular objects and numerous short filaments. These filaments were at first considered to

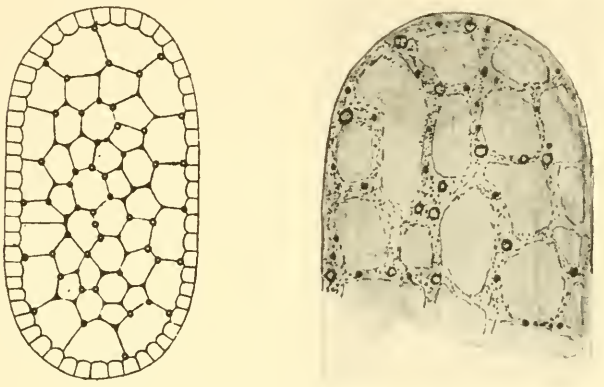

FIG. 51.

a.—*Achromatium oxaliferum.* Figure given by West and Griffiths as a copy of one of Schewiakoff's figures of *Achromatium*, in which is shown a marked distinction between peripheral and central cytoplasm.

b.—Figure of *Achromatium oxaliferum* as given by Virieux in which no such distinction between the peripheral and central positions is shown.

be short peritrich cilia arranged in the cell somewhat after the manner of the cilia arrangement in the Diatoms. Virieux found, however, that they are an artefact. The cell wall is lamellose, consisting of several firm layers with intervening layers which become gelatinous on the addition of carbolic acid. From its behaviour with certain chemical reagents West and Griffiths concluded that the cell wall is composed of a substance analogous to fungus cellulose.

The *protoplast* is uniform and composed of a reticulum evenly distributed throughout the cell. In the work of earlier

workers the protoplast of *Achromatium oxaliferum* is shown as two well-marked regions, an outer which was considered as the Rindenschicht (peripheral layer), and an inner region which was regarded as the Centralkörper (central body). Later workers consider that this distinction does not exist, and that the earlier workers made the same error as we have noted above in the investigations of *Chromatium Okenii*.

In the organism examined by West and Griffiths, by Virieux, and by the author, the protoplasm showed no such distinction (Fig. 51). The meshes are occupied each by a large globule, varying from 6μ to 10μ in diameter, of a calcium salt. These are steel blue in colour, and highly refringent. Outside the cell they crystallize as flat rhombohedra or rhombic prisms. West and Griffiths consider the salt to be calcium carbonate, but Virieux states that when treated with hydrochloric acid, it disappears without effervescence, and so considers it to be calcium oxalate.

Virieux's Investigation of Achromatium Oxaliferum.—This writer gives the name of *granules* to the calcium globules which fill the alveoles, and states that when they are dissolved, refringent particles which are neither chromatin nor metachromatic granules appear in the plasma. The metachromatic granules are soluble in dilute acetic acid, whereas the refringent particles are insoluble in that fluid. These particles are further distinguished by turning red when the cell is treated with haemalum, the rest of the cell being violet. He gives the name *corpuscles* to the refringent particles and considers that they are composed of sulphur, in spite of the fact that his microchemical reactions for sulphur did not give positive results. He bases his conclusion on the fact that they increase in number when the organism is supplied with sulphuretted hydrogen. They vary from 0·5μ to 2μ in diameter (Fig. 51*b*).

In addition, Virieux maintains that grains of *chromatin* are present which are so small that they are not visible unless the magnification is greater than 1000 diameters. The sum total of these particles is, according to him, the *nucleus*.

Achromatium mobile (Lauterborn), syn. *Microspira vacillans* (Gicklhorn) (see Fig. 16).

The general appearance of the organism is described on page 121. The cell is limited by a distinct membrane, which can be separated by plasmolysis, and is almost completely filled by two or a very small number of large globules. In addition, there are five to fifteen smaller globules. Gicklhorn considers the smaller to be composed of sulphur, but is uncertain of the constitution of the large bodies. Each of these appears to be formed by secretion into a previously formed vacuole, which is limited by a wall. The formation of a vacuolar wall is unknown in plant cells, and the observation needs confirmation.

The plasma is stated to be confined to the periphery of the cell, but there must be bands of plasma running across, otherwise it would be difficult to account for the vacuoles; there must be a plasma to form a matrix in which the vacuoles can form, for each must be sufficiently large to admit of the formation inside it of one of the "large bodies." The cell does not contain a well-defined nucleus.

Thiovulum Müllerl (Warming), Lauterborn.

This peculiar organism was investigated by Hinze (6) from material obtained from the Gulf of Naples (see Chap. VII.), and differs essentially from any of the organisms hitherto considered, by exhibiting *longitudinal* cell division. The cell is bounded by a delicate membrane, and the plasma consists of delicate strands which, however, are completely absent from the centre of the cell, which is occupied by a large oval vacuole. At the thicker end of the cell is an aggregation of plasma in which numerous sulphur globules are placed (Figs. 18–19).

In addition to sulphur, Hinze found large grey-green structures of fugitive existence which he regarded as a form of reserve material (Fig. 18). Further, he found in the plasma very fine granules which colour in the same way as the nucleus, and which also he regarded as reserve food.

Cell-nucleus.—The most remarkable feature in the cell is a body which when stained with Delafield's hæmatoxylin exhibits a coloured centrum. This organ is regarded by Hinze as the *nucleus*, and the coloured centrum as the *nucleolus*. Hinze further stated that the nucleolus breaks up into fragments before division and that nuclear division follows the longitudinal cell division characteristic of this organism. Hinze's figures are somewhat unsatisfactory, and confirmation of his interesting statements is required.

Thiovulum majus (Hinze).

The cell is ellipsoid in form, and possesses a sharply contoured membrane. Inside it, and penetrating in all directions, are delicate plasma strings. In some cells the plasma is massed at one of the ends, and a large vacuole occupies the greater part of the cell. Chief among these plasmatic inclusions are the sulphur globules, which are soluble in 90 per cent. alcohol, and insoluble in hydrochloric, nitric, and acetic acids. In the layer of protoplasm lining the wall are found dull green, round, oval, or sometimes angular plates, of varying size. They appear to increase in quantity as the sulphur contents decrease, and are not present in all individuals. Hinze regards them as reserve material, but of a different nature from that found in *Monas Mülleri*. They stain with Sudan, di-methyl-amido-azo-benzol, iodine and methylene blue; with Delafield's hæmatoxylin they stain a blue violet. The material is soluble in caustic potash, and also in a 1 per cent. solution of nitric, sulphuric, and hydrochloric acids, but is insoluble in acetic acid.

There are also in the plasma numerous small particles which Hinze names "chromatin granules," and which react in a similar manner to similar particles in *Thiophysa volutans* and *Beggiatoa alba* to which he has given the same name.

Chromatium Linsbaueri (Gicklhorn).

An actively motile pinkish-red organism, ovoid in form, and measuring up to 15μ in length. It grows in a pond in

the Epping Forest, near London, and in autumn imparts a red coloration to the water.

Outer protoplast.—The cell is apparently delimited on the outside by what appears to be a membrane, but as it reacts to stains in the same way as the rest of the cell, it must be regarded as the outer somewhat denser part of the protoplast. Its living nature is indicated by the attachment of the cilium to it ; if it were not living, one living part of the organism (the cilium) would be completely separated from the remainder. On physiological grounds this is impossible.

It is possible to detach the two protoplasts by treatment with a saturated solution of picric acid for two weeks, when the outer part expands, as shown in Fig. 52*b*. By further treatment with weak methylene blue, the connection of the cilium with the outer protoplast is made evident. Further, during this procedure the inner protoplast contracts slightly, and assumes a rounded shape, as is shown in Fig. 52 *b* and *f*, leaving the outer protoplast as a delicate loosely folded covering.

When the cell is pressed gently downwards, what appears to be a darker layer at the periphery of the outer protoplast disappears, an added proof that there is no real membrane at the periphery of the organism, and that what appears to be a membrane is due to the manner in which the light falls on the edge of the organism. The pressure of the cell causes a rearrangement of the light, and the " membrane " disappears.

The slime layer is from 1μ to 2μ in thickness. A distinguishing feature of the structure of this organism is the occurrence in the outer plasma of one or two gaps filled with slime (Fig. 52*a*, *e*, *f*). They occur in any part of the cell and may be together, or separated by the length of the cell. Although exceptionally there may be only one of these gaps, usually there are two, and apparently their number never goes beyond this. Each is about 4μ—6μ in diameter and oblate-spheroidal in shape (Fig. 52*e*). As at such parts of the cell the inner plasma is separated from the outside only by a layer of slime it is possible to cause the inner plasma to project through these slime areas by setting up differences of pressure inside the cell.

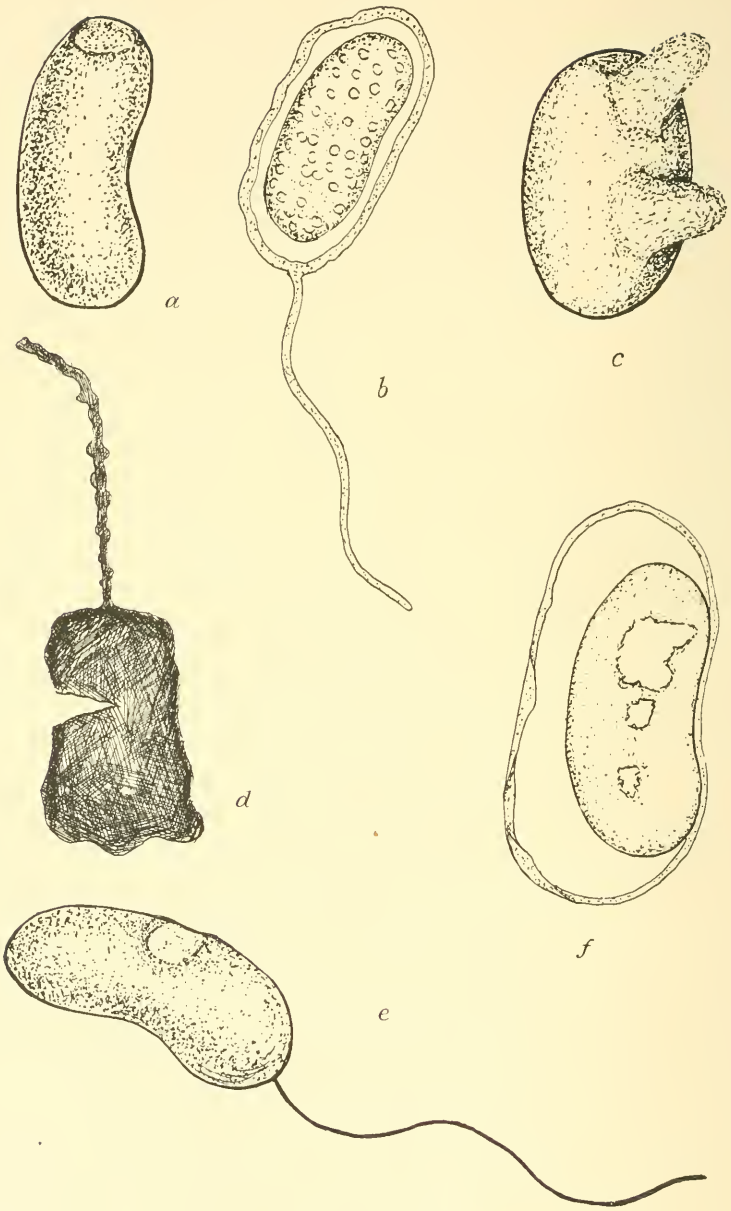

FIG. 52.

An example after this treatment is shown in Fig. 52*c*. Sometimes this can be accomplished merely by pressing the coverslip laterally downwards, sufficiently firmly to cause the cell to become somewhat flattened out. The effect of iodine on the cell is most marked, for it causes the outer plasma and its attached cilium to swell up in a very irregular fashion, and if the reagent be removed before it has affected the inner plasma it is possible to distinguish between the inner and the outer plasma with great clearness (Fig. 52 *b* and *f*). By allowing the cells to remain in picric acid for two weeks and then staining with dilute methylene blue the separation of the two plasmas may be made complete as is shown in Fig. 52*d*. In this in-

FIG. 52.—*Chromatium Linsbaueri* (Gicklhorn). × 1000.

a.—Unstained individual showing a single slime area at one of the ends.

b.—Cell treated with picric acid for two weeks, and then stained with dilute methylene blue. The outer plasma is shown with the cilium in attachment and the widely separated inner plasma has contracted and resumed the rounded form natural to the organism.

c.—Cell which has been treated to make a part of the inner plasma project through the slime areas.

d.—Cell treated with iodine, which causes irregular swelling of the outer protoplast. The irregularity in the swelling is noticeable even in the cilium.

e.—Normal organism showing its single polar cilium and one slime area.

f.—Cell in which the treatment with iodine has been advanced a step further than is shown in (*d*). The inner plasma has begun to disintegrate. The large irregular empty space marks the position of a slime area.

stance the outer plasma with its attached cilium has evidently been destroyed by the acid, but the inner plasma, protected probably by the slime layer, has not succumbed but has contracted and rounded itself within the destroyed outer plasma. It is noteworthy that when this takes place the contracted inner plasma appears to be bounded by a cell membrane. This effect is an optical one, as can be seen when the cell is pressed flat.

It is highly probable that the inner and outer protoplasts are connected by plasma strands, otherwise the organism could not function as a physiological unit. It is possible, however, that the slime layer may in reality be a somewhat tenuous

plasma, in which case it is not necessary to postulate the existence of connecting plasma threads. The inner protoplast is about 4μ in thickness, whilst the centre is occupied by a large vacuole. The inner protoplast is not reticulated, and frequently contains a large number of small round globules of calcium carbonate (see Fig. 30 A and B). When the organism is slowly rotating it can be seen that these bodies are confined entirely to the inner protoplast. As already stated, Gicklhorn suggests that this is an example of a new class of organisms which he proposes to name *lime bacteria*. All the round globules are not composed of lime, for whilst some (the lime globules) do not stain with methylene blue, others do take up the stain. The globules that take up the colour are probably reserve material. This is supported by the fact that their number fluctuates, suggesting that they are first stored, and then used up during metabolic processes.

There are also very small granules distributed in the cytoplasm but their nature is unknown.

The sulphur globules are distributed in the plasma and when present their number varies considerably.

The colouring matter is pinkish-purple in tint and diffused uniformly throughout the plasma, a detailed account of the pigment is given in Chap. XIII.

Summary and Conclusions.

The intimate structure of the cell in the sulphur bacteria shows the same primitiveness as the rest of the Schizophyta. There is less differentiation than is found in the cells of the higher Fungi, or of the Algæ. With one exception (*Beggiatoa mirabilis*), the outer envelope or "membrane" is plasmatic, and distinguished from the inner plasma only by its greater density. It takes up the same stains, and in the one species tested, namely, *Chromatium Linsbaueri*, is the base to which the cilia are attached.

Division throughout the group is by the simple fission characteristic of the Schizophyta. The cells when divided maintain connection for a period by a bridge of slime, but

later draw away, and effect complete separation. The transverse walls that are seen in such organisms as *Beggiatoa alba* and *Thiothrix* are not plasma-derived. They are transverse bands of slime that have formed *between* the dividing cells, and owe their origin to the transformation of the outermost layers of the cell into slime.

Slime Formation.—This is well developed in many of the sulphur bacteria, although when the organisms are actively motile its amount is very small. The slime, when formed, covers the whole organism with a closely folded mantle, which subsequently hardens. In *Thiothrix* the slime hardens early and forms a permanent sheath enfolding the inner cells, which continue to grow, and to be successively thrust out of the sheath. In *Beggiatoa alba* excessive sheath formation seems to occur only under unfavourable conditions. The production of slime by a mass of clustered cocci leads to the zoogloea condition, and when the cocci multiply inside the slime an appearance is presented of a totally different organism from that from which the cocci were derived. This has led, in the author's opinion, to the erroneous formation of new species, and even of new genera.

Slime formation in some cases has become fixed to the extent that the cells of an organism pass their whole existence inside a slime covering. This is the condition of such bacteria as *Thioploca*, *Thiopedia*, and *Thiodictyon*. It may be presumed that a continuance of this colonial habit would in time lead to the differentiation of the cells, and the evolution of a multicellular organism. Such an advance, however, has not been accomplished by any of the sulphur bacteria.

The Question of the Nucleus.—There is no readily demonstrable nucleus in any member of the sulphur bacteria. Two claims have been made, both of which need confirmation. It will not be possible to prove or disprove Virieux's claim until the behaviour of the small bodies, alleged to be the nucleus, is observed during cell division. Hinze's claim that a nucleus is present in *Monas Mülleri* rests on a somewhat sounder foundation, and if it can be confirmed that the body that he regards as the nucleolus breaks up into fragments during cell

division, and that such fragmentation is associated with cell division, his view would be considerably strengthened. This organism, however, is far from being a typical sulphur microbe, and in all probability should find a place among the *Flagellates*. It is noteworthy that even in such large cells as *Beggiatoa mirabilis*, in which, if present, the typical nucleus would be a prominent object, there is no trace of it.

The cytoplasm does not call for special mention, except to stress the fact that in no single instance is there a differentiation between a peripheral and a central cytoplasm based on a marked difference in the size of the vacuolar meshes.

Cell Inclusions.—Sulphur appears to be the inclusion which is common to all these organisms. As they flourish under marine, brackish, and fresh water conditions, it is probable that the total number of metabolic products is very large, and that the different organisms show considerable variety in this respect. The chemical analysis of the sulphur bacteria on a large scale has yet to be made.

Interesting inclusions are the lime granules of *Chromatium Linsbaueri*. In this organism also are small round bodies which may be stained with methylene blue, and which are probably protein reserve products. The minute granules that are found in *Beggiatoa mirabilis*, *Beggiatoa alba*, and *Thiovulum majus*, are probably of the same nature.

The core to the sulphur granules which was found in *Thioporphyra volutans* appears to be the same as the " sulphur builders " which Hinze found in *Thiophysa volutans*. It is probable that *in all sulphur bacteria the sulphur globule has an organic core*, and that it plays an important rôle in the metabolism of the sulphur bacteria.

The apparent secretion of the sulphur globule into a vacuole recalls the mode of formation of the spore in the genus Bacillus; see A. Meyer, and Ellis (1), and the formation of the spore in the genus *Sarcina* (Ellis (1)).

CHAPTER XI.

IRRITABILITY; INFLUENCE OF LIGHT; CHEMIOTACTIC PHENOMENA.

Irritability.

Introduction.—Irritability, or the response of a living organism to external influences, is characterized by a movement of an organism as a whole, or in part, either towards or away from the source of influence. Capacity for movement is one of the properties of protoplasm, and is as general among the lower plants as the lower animals. The higher plants are rooted by their habit to the soil, and so are bound by their physical conditions, but even they make a partial response to external influences. For example, when young cress seedlings are made to grow at the dark side of a room their stems assume a horizontal position in their endeavour to reach the light. Movements either of attraction or of repulsion may be caused by light, gravity, water, injury, and various chemical substances. The movement is usually, but not invariably, one that is distinctly beneficial to the plant. As an exception may be mentioned the movement of certain bacteria towards corrosive sublimate which is toxic to them.

Much has yet to be learnt about the response of the sulphur bacteria to external influences, but there are considerable data on the effect of light and of various chemicals in inducing movements. Some of the coloured bacteria of this group are probably the most sensitive of all organisms to light, and the response both of coloured and of uncoloured sulphur bacteria to certain chemical compounds is very marked.

Methods of Investigation.

Light.—The procedure in determining the influence of light is simple. The organisms are exposed to light of varying intensity and direction. By projecting a spectrum on to the organisms their movements through the different colours may be noted. In various simple ways it is also possible to expand or contract the length of the component colours of the spectrum, and to alter their intensity. In the examination of the effect of colour elaborate apparatus has been used which will be explained later.

Chemicals.—Pfeffer's Capillary Tube Method is used for the determination of the effect of chemicals on the sulphur bacteria. A piece of capillary tube of approximately 0·05 mm. bore by 1 cm. long is sealed at one end, filled with the salt solution under investigation, and completely immersed in the fluid containing the bacteria. If sensitive, the organisms move either towards the open end of the tube, or away from it. Some of the movement is due to diffusion between the two fluids, but its extent can be estimated by control experiments. The movements usually occupy one to several days before they are completed, and by a system of controls it is possible to allow for all other influences except that due to the fluid under investigation. By using a fluid which does not contain nutrient matter multiplication of the bacteria is avoided. The entrance of the bacteria into the capillary tube can be easily followed when viewed under the microscope.

Influence of Light on the Coloured Sulphur Bacteria.

The colourless sulphur bacteria are indifferent to light, whether they are cultivated in the dark or in the light. On the other hand, the coloured sulphur bacteria are extremely sensitive. Light affects plants in various ways. A distinction must be made between the *tonic*, the *directive*, and the *photosynthetic* effect of light. An organism like *Volvox*, for example, is influenced by light in three different ways. Light is necessary for its continued health, and so exerts a tonic influence; its direction of movement is determined by the line of incidence

of the light; and, finally, light supplies the energy for the carbon-assimilation which is characteristic of *Volvox* as of all green plants. The photosynthetic effect of light is not a phenomenon of irritability, and is discussed on page 204. There have been no specific investigations into the tonic effect of light on the sulphur bacteria, but it may be presumed that it operates in producing a state of well-being in the coloured sulphur bacteria, for, so far as is known, no multiplication and no pigment formation take place in the dark. It is impossible to separate altogether the tonic from the photosynthetic effect of light. The absence of colouring matter in the sulphur bacteria from which light has been withheld may be the cause of their lack of tone. It is responsible for their failure to multiply, and so it is impossible in many cases to state whether there is a tonic effect of light on these bacteria apart from the deleterious effect produced by the absence or loss of pigment formation. In some cases, however, a distinct tonic action of light may be noted. Thus it is shown in Engelmann's investigation that when motile coloured sulphur bacteria lose their motility through the withdrawal of light, they do not assume motility when they are once more exposed to light if they have been kept too long in the dark. This failure is to be attributed to the loss of tone by the withdrawal of light, and to the same cause must be attributed the fact that exposure to *constant* light intensity is injurious, for motile organisms stop moving altogether unless slight changes are made in the intensity of the light.

The most notable investigation on the reaction of the sulphur bacteria to light-intensity and colour is that of Engelmann (3).

Engelmann's Investigation: Effects of Changes in the Intensity of Light.

Engelmann used for his experiments a number of species which cannot now be identified. Among these was the very sensitive *Bac. photometricum* (Engelmann), which was possibly a pleomorphic form of *Lankesteron*.

The following is a summary of his conclusions :—

1. Within certain limits, a direct correlation exists between the velocity of the organism and the intensity of light. The dependence of one factor on the other is more marked at low oxygen pressures.

2. The movement completely stops when the organism is left in the dark beyond a certain period, and if kept in the dark beyond a further period it will not resume movement when again exposed to light.

3. Different species show responses which differ both in kind and in degree. An increase of intensity produces different rates of increase of velocity in different species. In some species an increase of intensity may even result in a decrease of velocity.

4. The change of velocity is influenced by the amount of H_2S present.

5. Exposure to light of constant intensity ultimately effects a stoppage of movement. When a quiescent organism, kept in the dark, is exposed anew to light, movement does not begin until a certain interval elapses. Engelmann called this delay in response *Photokinetic Induction.* The converse process, namely, the cessation of movement as a result of the removal of light also takes place after a slight time interval. This was named *Photokinetic After-effect.*

These delays in response to a given stimulus are comparable to the " presentation time " which elapses in roots of higher plants before geotropic curvature takes place.

Shock Movements (Schrenk Bewegungen).

The curious effect was observed in many of the sulphur bacteria that the sudden removal of light resulted in first a stoppage, then a slight backward movement, and finally a forward movement once more, but with diminished velocity. The organisms react as though they had received a slight shock from the sudden change. The shock becomes weaker on repetition, and is much less pronounced when it takes place in a medium rich in oxygen. The extent of the reaction differed

according to the suddenness of the change from light to darkness. Again all the individuals were not affected to the same extent, and some failed to respond altogether. Similar effects were obtained by causing the organism to traverse from one colour to another. If the intensity of light in the spectrum is great, the organism travels from one end of the spectrum to the other with undiminished vigour, but if the slit be made very narrow, shock movements are observed in passing from one colour to another. They were observed in the passage from beyond infra-red to infra-red; from infra-red to red; from yellow to red; from yellow to green; from green to blue; from blue to violet.

With one exception the reactions occurred when the organisms passed from a colour of greater to one of lesser wavelength. Engelmann did not apparently observe the effect of a passage from a colour at one end of the spectrum direct to one at the opposite end, but he noted that shocks were observed in passing from any colour to the dark, and that the shock was not so pronounced when the organism entered the dark from either extremity of the spectrum.

Shock Movements a form of Preservation: Valve Action.

Engelmann maintained that the shock movements exercised a self-preserving effect on the organisms, as their tendency was to prevent their passage into the dark. Thus an individual moving in the dark along the line *ab* (see Fig. 53) experiences no shock on reaching the light, but when it crosses the area of light and enters the dark zone on the other side a shock movement occurs which brings the individual back once more to the lighted region. The influence of light will now operate to prevent it once more taking up the path *ab* which would lead it into the dark. If the light is strong enough the reversed movement caused by the

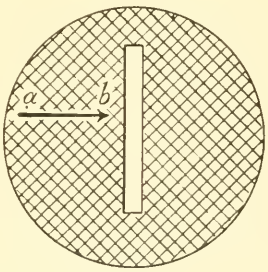

Fig. 53.

shock can be maintained and the individual remains in the light. When next it crosses the lighted area and once more reaches the dark zone on the other side a similar shock causes it to reverse again, thereby preventing its passage into the dark zone. The effect of the operation of these factors may be observed by covering a microscope field containing motile purple bacteria with a disc which darkens the whole field except for a restricted region. The organisms tend to collect in this region to the exclusion of the rest of the field. Engelmann compared these shock movements to the action of a valve, presumably because they exercise a certain measure of control on the direction of movement. The analogy is not a happy one, for the essence of valvular action is regulation, and there is no question of regulation of movements in the shock phenomena. It is doubtful whether the incidence of these movements is a factor of sufficient importance to affect materially the natural striving of the organism towards the light when it is about to pass from the light into the dark.

Molisch on Shock Movements.

Molisch experimented with *Rhodospirillum photometricum*,* an organism so sensitive that the effect of passing the hand between the light and the mirror produced a shock movement. Molisch was convinced that the colouring matter was closely concerned in the production of these movements, and that in a field of various microorganisms, including the purple bacteria, these could be recognized by their shock movements, even although the colouring matter was not present in sufficient quantity to be perceptible. He confirmed Engelmann's statement that shock movements are more pronounced in an atmosphere containing only a very small quantity of oxygen.

Distribution of the Purple Bacteria in the various Colours of the Spectrum.

Engelmann found that when a spectrum was projected on to the microscope field, the purple bacteria in that field

* This organism was probably some pleomorphic form of one of the better-known sulphur bacteria.

arranged themselves in greatest concentration in the *infra-red* (between lines * 80 and 90). There was a distinct but smaller concentration in the *orange-red* (between lines 54—64), and a still smaller gathering in the *green* (between lines 52—55), whilst a few also collected in the *blue-violet*. The organisms avoided the other colours. It is shown below (p. 200) *that the region of maximum concentration of these bacteria, namely the infra-red, is also the region of maximum absorption of energy*. Engelmann considered that the correspondence was significant, and that oxygen was liberated in that zone because photo-synthesis was carried on by the purple bacteria. Here, however, different wave-lengths were utilized for the absorption of light energy. The significance of these results will be discussed later.

Effect of Colour on the Absorption of Light.

In order to examine the absorption spectrum of the pigment found in purple bacteria, it is necessary either to prepare an emulsion of the organisms in water, or to extract the colouring matter with a suitable solvent. An extract with chloroform or carbon bisulphide is purple in colour; with ether or alcohol it is yellow or yellowish-green.

Engelmann used an aqueous suspension of *Bac. photometricum* and determined the exact amount of absorption at different wave-lengths by the use of Langley's Bolometer.†

Figures on the following page show the results obtained by him.

λ gives the wave-length of the different colours.

E gives the percentage of energy that is *transmitted* after the light has traversed the coloured fluid.

* See page 200, footnote.

† The *Bolometer* is an instrument used for estimating the total energy contained in any part of the spectrum. As it travels along the spectrum it picks up the energy, with the result that it causes an alteration in the resistance of an electrical wire with which it is in attachment. The amount of alteration in the resistance is measured by a galvanometer.

	λ.	E.	
infra-red	1·60*	94·4	
	1·40	94·8	
	1·00	78·3	
	·95	69·5	
	·90	44·2	
	·85	2·91	→ 1st max.
	·80	30	
	·70	69	
	·68	77	
	·66	80	
	·64	84	
	·62	77	
	·60	40	
	·59	27	⟶ 2nd max.
	·58	28	
	·57	28	
	·55	18	
	·53	9·5	
	·52	40·5	
	·50	9·0	
	·48	10·5	
	·46	12·0	
	·44	17·0	

Thus the point of maximum absorption is at wave-length 85 in the infra-red, which is beyond the end of the visible

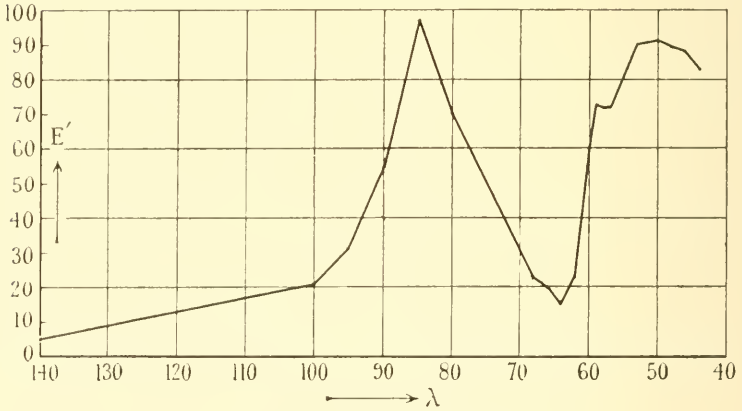

Fig. 54. (After Engelmann.)

spectrum. A second maximum, and a third, appear at 59 and at 50—52, in the orange and green-blue, respectively.

In the accompanying graph (Fig. 54) the ordinate gives the energy absorbed (E′). In Engelmann's table the figures give the proportions transmitted (E).

*The figure 1·60 = 1·6 × 10,000 Ångstrom units. $\lambda = 16{,}000 \times 10^{-3}$ cm.

Liberation of Oxygen by the Purple Bacteria on Exposure to Light.

Whilst direct proof of the liberation of oxygen is still not available, strong presumptive evidence of its liberation is supplied by the following facts, which were elicited by Engelmann.

1. A colony of sulphur bacteria in the zoogloea condition was introduced, together with a strongly aerobic spirillum, into a drop of water. The spirilla collected round the colony if the drop was poor in oxygen, but did not do so if the drop was well aerated. If the space surrounding the drop was filled with hydrogen the spirilla showed a greater tendency to collect around the zoogloea.

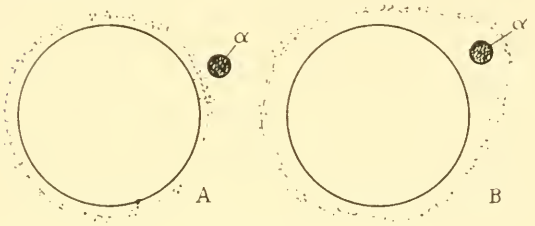

Fig. 55.

2. Variations in the pressure of oxygen were correlated with variations in the activity with which the movement of the spirilla towards the zoogloea took place.
3. In one observation of a drop of water which had been kept for some time in the dark and then brought into the light, a number of spirilla were observed in position round the circumference of an air bubble, and near the bubble there was also a purple organism (*Monas vinosa*) (marked α in diagram). When the drop was rich in oxygen the purple organism had no particular attraction for the spirilla (Fig. 55A), but when it was poor in oxygen they distributed themselves as shown in the B position.
4. Many of the actively motile sulphur bacteria are attracted by oxygen. If a drop of water containing

the bacteria is placed under a coverslip and left in the dark, the organisms take up a position varying from ½ mm. to 2 mm. from the edge. If the drop is now exposed to the light, the bacteria scatter in all directions. This is consistent with the explanation that they had produced oxygen in the light, so that their movements were less restricted.

5. A fluid containing purple bacteria was placed in a glass tube 5 cms. high, and exposed in a vertical position. After five months the fluid was colourless except at the bottom, where there was a layer of the bacteria 2 mm. high. By attaching the tube to a hydrogen-generating apparatus the pressure of oxygen was diminished. If this apparatus was now placed in the dark the organisms scattered throughout the field, but if in the light they collected at the bottom. In this case the inference is that in the light so much oxygen is developed that its concentration is greater than the optimum for these bacteria, and so they move as far as possible from the surface. The effect in this experiment of introducing oxygen is to cause repulsion of the bacteria, a result which is the opposite of that obtained in the previous experiment. It must, however, be borne in mind that the sulphur bacteria are attracted to oxygen up to a certain concentration, and therefore it must be presumed that in the previous experiment the concentration of oxygen was below the optimum, whilst in the present experiment that point had been passed.

The facts, however, have been challenged by subsequent investigators. Molisch (3), working with pure cultures of purple bacteria found that oxygen was not liberated in sufficient quantity to collect in fermentation tubes. Further, he found that the purple bacteria had no noticeable effect on motile bacteria sensitive to the presence of oxygen, when such bacteria were cultivated in association with purple bacteria.

He also stated that the oxygen which is necessary for the manifestation of phosphorescence by phosphorescent bacteria

cannot be supplied by growing the purple bacteria along with these organisms.

Both Molisch and Winogradsky have concluded that the oxygen in Engelmann's experiments was derived from green organisms accidentally present in the medium. It is evident that the whole subject must be reinvestigated, before Engelmann's observations can be accepted. In his favour it may be mentioned that Molisch's results were all negative, whilst those of Engelmann were positive. In one experiment at least the effect of contamination with green algæ can have no part; in the third experiment mentioned the bacteria are shown clustered round a single individual of the purple bacteria, and here there can be no question of the interference of green organisms.

Relationship of Light to the Growth of the Purple Bacteria.

Engelmann sought to establish a relationship between growth and light from the following experiment. When tubes containing sea-wrack infected with purple bacteria are left in the dark they remain colourless. When other tubes of a similar kind are exposed to light the colour deepens. When now the first set is exposed to the light the colour develops after only a few days, and if the second set is now placed in the dark the colour disappears. It is possible to put another interpretation upon these results, for the mass of individuals of the purple bacteria may remain stationary in point of numbers, and develop or lose colour according to whether they are placed in the light or the dark. Thus proof is supplied of the dependence of colour on light but not necessarily of the measurement of growth by colour. However, Winogradsky (1), Molisch (3), and Skene have shown conclusively on other grounds that the growth of these organisms is dependent on light. The dependence is established by the fact that the spread of the purple colour to cover fresh material readily takes place in the light but not in the dark. Skene cultivated purple bacteria in different coloured lights and

showed that the relative efficacy of daylight, red and blue light was about as 6 : 3 : 1 respectively.

The Function of Light : Photosynthesis.

It is universally agreed that light is essential to the sulphur bacteria, but there is considerable diversity of opinion as to its precise function. It has been maintained by Engelmann and others that light is a source of energy for an anabolic process comparable to the photosynthesis of green plants. It will be recollected that the chlorophyllous plant tissues utilize solar energy for the synthesis of carbohydrates from carbon dioxide and water. During the process oxygen is liberated as a waste product.

Now Engelmann has demonstrated that the purple bacteria liberate oxygen in the light, and it is from this that he deduces that a similar photosynthetic process takes place in them. Both Engelmann and Molisch have made the liberation of oxygen the test of photosynthesis, whereas the essential proof is the demonstration of the synthesis of more complex from less complex compounds. Engelmann proclaimed the occurrence of photosynthesis chiefly because of the liberation of oxygen, whilst Molisch denied its occurrence because he considered oxygen was not liberated. It is possible to imagine a photosynthetic process in which oxygen is not liberated.* Hence as neither advanced proof of *synthesis* the question must still be regarded as an open one. Subsequent opinions on the occurrence of photosynthesis have mainly centred on the question whether oxygen is liberated, and are therefore not relevant.

Whilst definite proof of photosynthesis is not available the following facts form strong indirect evidence of its occurrence in the purple bacteria.

*As an analogous case may be mentioned the fact that respiration is in essentials the katabolism of complex compounds, and not only may the process be imagined without the intake of oxygen, but there are actually organisms (the anaerobic bacteria) in the respiration of which oxygen is not taken in.

1. The indispensability of light for the growth of the purple bacteria, and for the development of the colouring matter.
2. The fact that carbon dioxide is the source of carbon of these organisms.
3. The fact that the part of the spectrum which absorbs the greatest amount of energy is also the part at which it is highly probable oxygen is evolved.
4. The known use of the light in this manner in green plants.

Molisch (3) has advanced the opinion that light is used to break up the organic matter which he claims is absorbed by the purple bacteria. He brought no experimental evidence in support, and the opinion is evidently unsound, for the purple bacteria do thrive in the complete absence of organic matter, as has been shown by the investigations of Skene, Bavendamm, and others, and the necessity of light is as great in the absence as in the presence of organic matter.

If photosynthesis does occur in the purple bacteria it is of special interest, for whilst in the green plants the red and the green constituents of light are the most effective, in these bacteria the most active rays are in the infra-red. It is of interest that different organisms use light of different wave-lengths and suggests that light of other wave-lengths may be utilized by other organisms of whose physiology we have at present little knowledge.

The Directive Effect of Light.

Engelmann stated that light exerted no directive influence on the purple bacteria, but Winogradsky maintained that there was such an influence, because the purple bacteria collected on the side of the culture vessel which was turned towards the light. This statement is, however, incomplete, for it is a matter of common observation that in intense light they collect with equal readiness on the side removed from the direction of light. Also it may be pointed out that the appearance of colour does not alone indicate movement, for it may appear as a result of the development of pigment

in organisms that were hitherto colourless or in which the colour was feebly developed. In such a case there would be a development of colour without mass movements of the organism in any particular direction. Hence colour may develop on the side of the culture vessel towards the light because the individuals on that side have been stimulated by the light to develop the pigment to a stronger degree. Winogradsky also attributed the shock movements to the directive effects of light, but the statement was made from general observation, and the experimental data given above do not support this view. Beijerinck investigated a species of *Chromatium* and found that it swam towards the point of maximum light intensity. This observation is not correct, for, as already stated, in bright light the bacteria collect on the side of the culture vessel which is away from the light. Beijerinck's statement would be true only if he experimented in light of low intensity. Molisch (3) came to the conclusion that light exercised no directive influence on the purple bacteria.

The whole subject needs reinvestigation.

Summary of the Effects of Light on the Purple Bacteria.

The *tonic* effect is indicated by the necessity of light for the development of colour, and for the growth of the bacteria, and by the fact that the resumption of movement in the light is not possible if the organisms are previously kept too long in the dark. Also, the need of a slight change in the intensity of light to keep the bacteria at their highest speed is indicative of this tonic effect.

The *directive* or *phototactic* effect of light on organisms in general is of two kinds. There is a directive effect which makes the organisms change the inclinations of their directions so as to bring them either nearer or farther away from the source of light. There is no evidence that the purple bacteria respond in this way. On the other hand, there is ample evidence that changes in the intensity or in the colour of light produce the second effect, namely, a complete

reversal in the direction of movement. The conditions determining such reversals have been described above.

The *photosynthetic* effect of light has not been proved. Reasons have been given for the conclusion that in all probability some form of synthesis does take place through the agency of light.

The Effect of Chemical Substances in Changing the Direction of Movement.

Miyoshi's Experiments.—The table on next page gives the results of this investigator with Pfeffer's Capillary Tube Method.

The absence of controls in these experiments somewhat impairs the value of these results, but there is no doubt of the sensitiveness of the bacteria to peptone and flesh extract with which the most marked results were obtained.

Bengt Lidforss found that even very minute quantities of ethyl alcohol attracted certain bacteria. He also found that a colourless sulphur spirillum was attracted by a dilute solution of sulphuretted hydrogen, but that this compound was poisonous in concentrated solutions. It is inferred that the organism was presumably repelled by more concentrated solutions. A substance may, however, be poisonous to bacteria and yet exercise attraction for bacteria, as for example corrosive sublimate.

Sodium thiosulphate was also found to attract bacteria in dilute solution.

Molisch's Experiments.—The results obtained by Molisch gain an added value from his rigorous use of controls. The methods of experimentation were the same as those used by Miyoshi. He established the important point that different purple bacteria *may react differently to the same reagent.* Thus *Rhodospirillum giganteum* is attracted by weak hydrochloric acid, whilst *Rhodospirillum photometricum* and *Chromatium* are repelled. Again, *Rhodospirillum giganteum* is sensitive to carbon dioxide, hydrochloric acid, dextrin, sucrose, and peptone, whilst *Chromatium* is in-

MIYOSHI'S TABLE.

Name.	Concentration of Solution per cent.	Result.	Remarks.
Sulphuretted hydrogen	Weak Strong	Indefinite +	Results sometimes negative : must be regarded as inconclusive.
Pot. nitrate	0·3 Other strengths	+ Indefinite	Slow entrance : crowded mass of bacteria moved slowly inwards at rate of 1·5 mm. per hour.
Am. tartrate	0·5	+	Bacteria appeared at mouth, then moved slowly inwards. Many remained outside. After a month in the dark inside the tube, the bacteria moved still farther up when exposed to the light.
Pot. dihydrogen phosphate (KH_2PO_4)	0·3 0·8	+ + + Doubtful	
Sod. chloride	0·3 0·8	+ −	
Am. chloride	0·3 0·3	+ −	
Am. sulphate	0·3	+	
Mg. sulphate	0·3	−	
Peptone	0·5	+ +	
Flesh extract	0·5	+ + +	
Sucrose	0·5	+ +	
Glucose	0·5	+ +	
Asparagin	0·5	+ +	
Glycerine	0·5	+ +	
Baking soda	0·3	−	
Pot. chlorate	0·3	−	
Alum	0·3	−	
Acetic acid		+ +	
Lactic acid		+ +	
Mono-, di-, and tri-hydric alcohols of fatty series		+ +	
Aldehydes		+ +	
Ketones		+ +	
Tri-hydric alcohols		−	
Tetra- and Penta-hydric alcohols		O	

The + sign indicates a movement towards, the − sign a movement away from, the source of stimulus.

different to all these substances. Further, *Rhodospirillum giganteum* is attracted, but *Chromatium* repelled, by sulphuretted hydrogen (concentration not stated). He also established the important fact that *for every reacting substance there is a*

particular range of concentration within which the organism is sensitive. Above this range the fluid may injure, or even destroy, the organism, whilst below this range there is no reaction. These observations are in general agreement with the results of similar tests on the effect of chemical substances on spermatozoids, and small organisms. Molisch's results are tabulated below. The organism used for experimentation was *Rhodospirillum giganteum.* In the third column the signs +, —, and 0, have been inserted from Molisch's description, and were not so set down by the investigator himself.

Name.	Percentage Strength of Solution.	Reaction.	Remarks.
Carbon dioxide		+ + +	The bacteria stream in and remain inside for some hours, after which they stream out again until the distribution is uniform throughout.
Hydrochloric acid	0·005	+ +	*Rhodosp. photometricum* is negative at concentrations of 0·01—0·001 per cent.
	0·01	+ +	
	0·1	—	
	Greater concentrations	—	
Sulphuric acid	0·005	+	*Rhodosp. photometricum* is indifferent at all concentrations.
	0·0025	+	
	0·01	0	
Nitric acid	0·01	+	
	0·1	—	
Acetic acid	0·01	+	Reactions variable, sometimes caused repulsion.
	0·005	+	
Caustic potash	0·1	—	Same result with *Chromatium.*
Potassium chloride	1·00	+	*Chromatium* weakly positive.
Dextrin	1·0	+ + + +	*Chromatium* weakly positive.
Cane sugar	1·0	+ + +	*Chromatium* indifferent.
Peptone	1·0	+ + +	*Chromatium* indifferent.
Sulphuretted hydrogen	not given	+ + +	*Chromatium* (marine species) indifferent.

Irritability and Environment.

It was maintained by Kniep, who experimented with a colourless sulphur organism and with *Spirillum rubrum*, that the reaction of an organism to a particular salt is influenced by the presence of other salts. Thus *Spirillum*

rubrum is sensitive to sodium chloride, and also to ammonium chloride. But if, in Pfeffer's experiment, the water in the capillary tube contains both 1/100 sodium chloride and 1/100 ammonium chloride, and if the bacterial suspension outside the capillary tube contains 1/100 sodium chloride, there is no attraction. The presence of sodium chloride has destroyed the efficacy of ammonium chloride. Similarly ammonium chloride neutralizes the efficacy of sodium chloride. The same held true for other combinations of salts, for example, potassium sulphate and ammonium sulphate.

Buder's Researches.

(*a*) *Ciliary Movements of the Sulphur Bacteria.*—Very interesting results were obtained by this investigator in

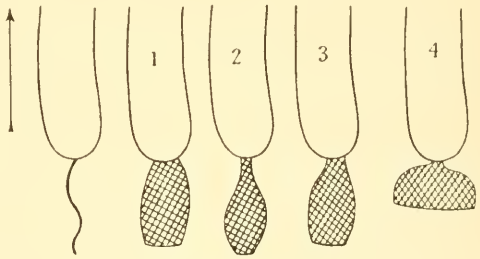

Fig. 56.—Positions of the cilium of a spirillum moving forward with the cilium attached behind. For explanation see text.

his examination of the cilia and the movements of *Thiospirillum jenense* (see p. 161), and a species of *Chromatium*. Buder found that the single cilium shows a rotary movement. The positions of the cilium when the organism is moving forward with the cilium behind are shown in Fig. 56.

The shaded parts show a section of the space enclosed during the revolution of the cilium. It will be seen from the diagram (Fig. 56) that the shape of this space is, during movement, continuously undergoing alteration, and that the alteration takes place in a definite order. In No. 4 of the series the cilium is rotating almost at right angles to the spirillum. This is the position which it assumes prior to that indicated in

Fig. 57. The cilium then revolves round the hinder part of the body and propels the spirillum in the reverse direction.

(*b*) *Reaction to Light.*—The reaction following the exposure of an organism to light of a given intensity depends on the intensity of the light to which the organism was previously exposed. There may be, for example, a reaction when the light intensity is increased from 18 units to 20 units (units arbitrarily chosen), but not from 40 to 42, although the difference in intensity, namely, 2 units, is the same. Before a reaction due to a change of intensity occurs, the new intensity must be a certain *percentage greater than the original intensity*, and so the greater the original intensity the greater the number of units which must be added to raise the percentage to the required point. The susceptibility of the human eye to a change in the intensity of

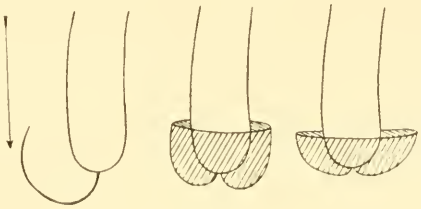

Fig. 57.

light is formulated in Weber's Law, which states that before the eye can appreciate a change in the intensity, the increment of light units must bear a certain ratio to the original intensity, and this ratio is independent of the value of the intensity. Weber's Law does not hold for the reactions of *Thiospirillum jenense* to light, for whilst in some cases an increase of 10 per cent. on the original intensity was required to produce the effect, in other cases as much as 25 per cent. was necessary. Again, according to Buder, the changes in the direction of movement in response to changes in the intensity of light are not invariably as sudden as was claimed by Engelmann. The length of time required for the completion of the reaction depended on the velocity of the organism at the moment, the length of time taken by the light to change its intensity, and on other factors, so that under some conditions quite an

appreciable interval of time elapses before the reaction is completed.*

(*c*) *Experiment on Shock Movements.*—An interesting contribution to our knowledge of shock movements was made by Buder by an experiment which is illustrated in Fig. 58. In No. 1 a spirillum with a single polar cilium is shown moving towards a dark patch. Nos. 2, 3, and 4 show its further progress. At No. 4 three-quarters of the organism, but not the sensitive region, which is at the base of the cilium, is inside the dark patch. In Nos. 5, 6, 7, and 8, the dark patch is made to move in the

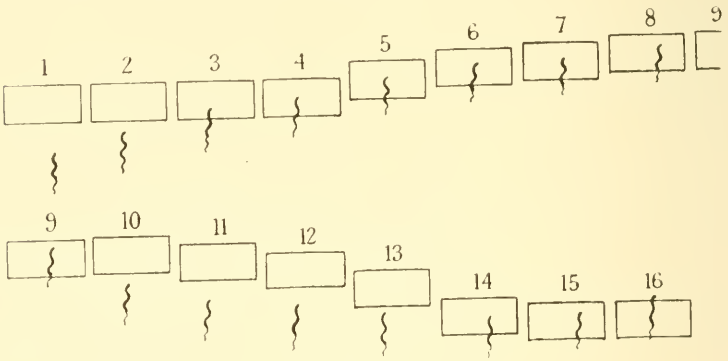

Fig. 58.

same direction as the spirillum, to prevent the sensitive zone from entering the dark patch. No shock movement occurs, because the sensitive zone is still outside the dark patch. At No. 9, however, the sensitive zone is shown for the first time inside the dark patch. The result is shown in No. 10, a shock movement having caused a strong recoil into the light. Whilst the spirillum is recoiling the dark patch is made to follow at a greater rate than the spirillum, and thus to overtake the organism. This phase is shown in Nos. 11-15. At No. 15 the dark patch overtakes the sensitive zone. The result here was that the organism executed another shock movement, which caused it to move forward again even although by so doing it moved still farther into the darkness. Hence shock

* The author considers that only failure will result from the attempt to express vital phenomena in terms of a strict mathematical law.

movements do not invariably have the effect of preventing the organism from moving into the dark.

(*d*) *Localization of Area of Sensitiveness.*—Buder has determined the part of the organism which is sensitive to light by the following experiment. A microscope field containing *Thiospirillum jenense* was completely darkened except for a small rectangular area. By an ingenious arrangement this patch of light could be set in any part of the field, and made to move in any part of it. In the illustration two spirilla (A and B) are shown (see Fig. 59). In B the cilium is in front, in A it is behind. In one experiment the lighted patch was made to move to the left at a greater speed than that of the spirilla moving in the same direction, with the result that the line CD overtakes the spirilla from behind. When this line overtakes an organism like A with the cilium behind, the response is sooner than when it overtakes one like B with its cilium in front. But in both cases the response, which in this case is a faster movement in the same direction, follows the passing of the line CD over the base of the cilium. Buder thus demonstrated that the base of the cilium is the seat of sensitiveness to changes in light intensity.

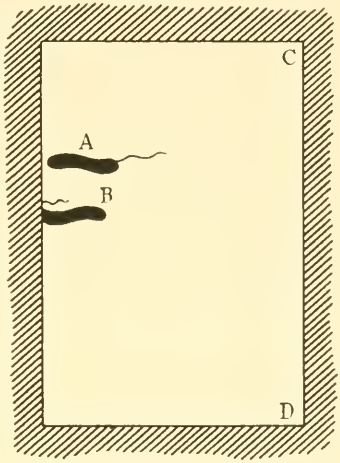

FIG. 59.

INTERPRETATION OF RESULTS.

The experiments which are illustrated in Figs. 58 and 59 show that under normal circumstances shock movements are such as bring the organism back into the light when it enters the dark, or when a dark patch passes over it. The circumstances were exceptional in the experiment illustrated in Fig. 58, and

cannot therefore be regarded as typical, for here a second shock movement was superimposed upon the organism before it had recovered from the effects of the first shock movement. Buder's own explanation is probably correct. When an organism passes into the dark it recoils in order to get back into the light, the mechanism of the process, for *Thiospirillum*, being the rotation of the cilium round the hinder part of the organism. It considers, as it were, the end in view, and if this can be attained without a shock movement, then such a movement does not take place. Buder thus regards the laws of shock movements as aspects of a wider biological law, operating to preserve the organism from destruction. Whilst in agreement with the general view, it must be added that all the details of his experiments do not lend support. Thus in the experiment illustrated in Fig. 58 the second shock movement which propelled the organism farther into the darkness should, according to Buder's view, not have taken place, for it was one which was injurious to the microbe. As this particular movement, however, was made under abnormal circumstances, its significance cannot be unduly stretched, particularly as there are other determinants of movement besides these shocks. Proof is still wanting, however, of the withholding of a shock movement when the withholding would be advantageous to the organism. It would, however, be difficult to stage the conditions for such an occurrence. Another detail which lacks conformity with Buder's view is the fact that all the individuals did not exhibit the movement when they passed from the light into the dark, a fact which seems to diminish their importance as vital factors in the life of the organisms. Here again it would be easy to imagine the prevention of the movement by the intervention of other factors. The conditions of movement are similar to the conditions determining any other physiological function. It takes place when several contributory factors are working in unison. A general review of the facts leads to the conclusion that shock movements are broadly of a protective nature, but that as movements are conditioned by several factors, the incidence of a shock does not invariably produce a protective

movement because of the contrary influence of other factors under abnormal circumstances.

Jennings in his description of the movements of the *Flagellates* states that these organisms have two movements, one the normal, and the other, which is brought into play only when the organism is abnormally placed. The idea seems fanciful.

CHAPTER XII.

THE MECHANICS OF CILIARY MOVEMENT. THIONIC ACID BACTERIA. THE PHYLOGENY OF THE SULPHUR BACTERIA.

The Mechanics of Ciliary Movement.

Spirilla exhibit both rotatory and translatory movements. These result from the movements of the cilium which in the spiral sulphur bacteria assumes the helicoid form. It is possible by a mathematical treatment of the forces which come into action when this kind of cilium is set in motion, to show that the organism must necessarily exhibit translatory and rotatory movements.

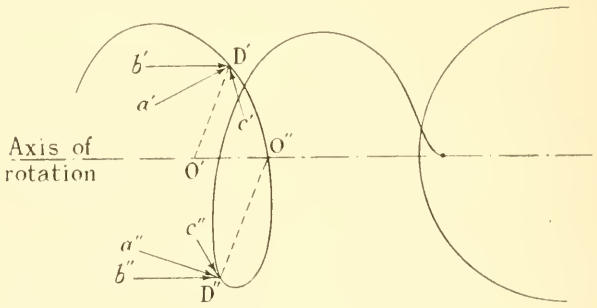

Fig. 60.

In Fig. 60 is shown a helically wound cilium rotating in a clockwise direction.

The cilium, by its whipping action, leads to a forward force on the spirillum, which may be considered the resultant of a fluid pressure.

The forces on each point on the cilium system can be analysed. Suppose D′ to be any point in the system. Let

$a'D'$ represent the force on D′ due to the reaction of the liquid to the motion of the cilium. This force is normal to the axis of the cilium and tangential to the cylinder of rotation, and may be divided into two components, one $b'D'$ along the axis of rotation, the other $c'D'$ in a direction perpendicular to the plane of this axis and O′D′. Similarly at any other point D″ the force may be split into the two components, $b''D''$ and $c''D''$.

The sum of the component forces $b'D'$, $b''D''$, etc., for every point on the cilium may be represented as BD, such that

$$\Sigma bD = BD.$$

Similarly, the sum of the tangential components $c'D'$, $c''D''$, etc., for every point on the cilium may be represented as CD, such that

$$\Sigma cD = CD.$$

The latter forces have a moment about the axis of rotation which tends to produce rotation of the body about this axis. Their summation gives a resultant couple F × MN (Fig. 61), such that

$$cD \times OD = F \times MN.$$

Consider the effect of these two systems of forces on the body of the spirillum (Fig. 62).

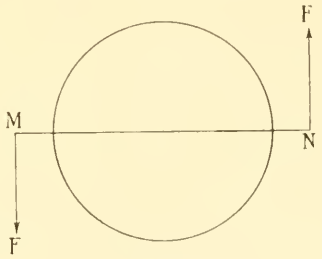

FIG. 61.

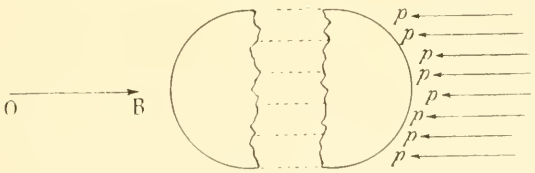

FIG. 62.

The resultant $\overline{OB}$ represents the total effective force acting upon the body from the water, probably through slight pressures established by the motion given to the water as a result of the whipping action of the cilium. The motion

gives rise to head resistance and frictional forces, p, the magnitude of which are dependent upon the speed of the body through the liquid. These tend to resist the motion and act in the opposite direction.

Then $\overline{OB} = Mf + kv,$

where M = mass of body,
f = acceleration,
k = a constant,
v = velocity.

The above equation shows that any increase in the velocity will produce a corresponding increase in the resistance. Movement will be uniform after a balance has been attained between the applied force and the opposing frictional force.

$$\overline{OB} = kV$$

where V is the ultimate velocity.

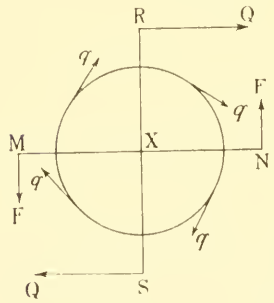

FIG. 63.

Let us now consider the conditions determining the rotational equilibrium (Fig. 63).

The couple F × MN tends to turn the body of the organism in the direction opposite to that in which the helix is turning. The body will set itself to counteract this force, and in consequence a redistribution of forces from the water will follow until the couple is balanced. This presumably cannot be accomplished in a straight cylindrical body with very soft membranous outer layers without altering its shape, and so if the spirillum is at rest it will at once assume the spiral form on the resumption of movement. The forces which here come into play may be illustrated by the following example. If the elbow is placed on a table while a long stick is held between the fingers and set into a whipping action, it will be found necessary to counteract the resulting torque reaction by offsetting the elbow to some extent in relation to the shoulder. Any increase in the force of the whipping, or in the addition of more weight to the stick will result in a corre-

sponding increase in the offset of the elbow. This gives us a possible explanation of the observed behaviour of the spirillum when in motion. This force is not exerted when the spirillum is at rest. The natural condition of a spirillum at rest is the straight rod shape, and many are observed to have this shape ; but we may suppose that the continuous assumption of the spiral shape when in motion, accompanied probably by a very slight increase in the outer membranous layers, has made most of the organisms retain the spiral form permanently.

When the ciliary impulse has been communicated to the body of the organism, and the latter has assumed the spiral form, there will be a similar torque between the body of the organism and the surrounding liquid medium, and the extent of this reaction will probably control the degree of spiral formation. As the body of the spirillum moves through the medium the reaction pressures of the liquid upon the organism will have a resultant which will be tangential to the body, and so a rotatory movement will be imparted to the body in addition to its translatory motion.

It is clear from the above dynamical treatment of the subject that a helicoid movement of the cilium ought to produce translatory and rotatory movements in the spirillum. As such movements do, in fact, take place in the spirilla, the observations of the helicoid whiplike action of the cilium described by Buder (p. 210) are supported.

The following hypothesis is suggested in explanation of the ciliary movement. The impulse, a vital action, comes in the first case from the end of the body to which the cilium is attached, and is similar in its nature to the elbow action in the above experiment. The vital action is communicated to the cilium, which then takes up the rotating helicoid form, thereby giving to the organism a rotational and translatory movement.

The following facts lend support to this hypothesis :—

1. The cilium is not an independent unit, but is in protoplasmic union with the cell.

2. It has been proved that the sensitive seat of the organism regulating movements in response to changes in the intensity of light lies at the end of the body to which the cilium is attached.

3. It is frequently observed that spirilla at rest are straight, and assume the helicoid form only after the organism has begun to move.

It is not possible at present to state whether the vital action which brings the cilium into the helicoid form is preceded by a circular movement of the end of the body which *ex hypothesi* is the seat of movement.

The Thionic Acid Bacteria.

Introduction: Methods of Culture.

The thionic acid bacteria, like the sulphur bacteria in general, oxidize sulphur compounds, but they differ from the sulphur bacteria in that they do not store sulphur in their cells. Strictly speaking, therefore, they are not sulphur bacteria, but it is appropriate to consider them briefly here.

These organisms oxidize thiosulphates to tetrathionic and sulphuric acids. They were first isolated by Nathansohn from a crude culture of organic debris and calcium sulphide in sea-water, prepared for the investigation of sulphur bacteria. Nathansohn isolated instead small actively motile thionic acid bacteria, which were readily found in the white sulphur surface scum.

He recommended the following medium for their culture:—

$NaCl$,	3·0 per cent.
$MgCl_2$,	0·25 ,,
KNO_3,	0·10 ,,
Na_2HPO_4,	0·50 ,,
$MgCO_3$,	Excess.

When this mixture was inoculated with mud containing these organisms a white scum appeared on the surface after a few days. This consisted of oily amorphous sulphur in the substance of which various bacteria were found. Among them were the thionic acid bacteria, which were isolated without difficulty. According to Nathansohn the reaction which takes place is as follows:—

$$3Na_2S_2O_3 + 5O = Na_2S_4O_6 + 2Na_2SO_4.$$

Their development is possible only when the CO_2 of the atmosphere is freely admitted; or, failing this, a free supply of carbonates. Nathansohn considered that they were not able to utilize the carbon of organic matter.

These organisms were also isolated by Beijerinck (5) from material taken from the sea on the Dutch coast, and cultivated in fresh water. The medium used by him was made up as follows :—

$Na_2S_2O_3 . 5H_2O$,	0·5 per cent.
$NaHCO_3$,	0·1 ,,
K_2HPO_4,	0·02 ,,
NH_4Cl,	0·01 ,,
$MgCl_2$,	0·01 ,,

A culture made up of these ingredients, and inoculated with the appropriate material, became covered with a film of sulphur in which were swarms of bacteria, including the thionic acid bacteria. These were isolated and grown in pure culture. The chemical reaction was stated to be as follows :—

$$Na_2S_2O_3 + O = Na_2SO_4 + S.$$

The sulphur was not deposited inside the cell, but in the surrounding medium. In a similar fashion, though with greater difficulty, it was found possible to oxidize tetrathionic acid with these organisms, according to the following reaction :—

$$Na_2S_4O_6 + Na_2CO_3 + O = 2Na_2SO_4 + CO_2 + 2S.$$

Pure cultures were obtained by adding the appropriate amount of agar-agar to the fluid culture, and plating in the usual manner. The colonies which form on the plates are distinguished by the large amount of sulphur which collects on their surfaces.

The thionic acid bacteria have been placed by Beijerinck in one genus, which he names *Thiobacillus.** One of them, *Thiobacillus thioparus*, is made up of small thin rods, 0·3—0·5μ in length, and is very motile. It is non-sporing. Another somewhat similar form has been named *Thiobacillus denitrificans.*

* See page 226.

When first investigated, the thionic acid bacteria were believed to be obligate autotrophs, obtaining their carbon from the carbonic acid of the atmosphere, and their nitrogen from nitrates or ammonium compounds. It was emphasized that the source of their energy was the oxidation of thiosulphate to the normal sulphate. Later investigations have not borne out these statements. Thus Issatchenko and Salismowskaja found that not only inorganic salts like ammonium chloride, ammonium phosphate, and potassium nitrate, but also organic compounds like asparagin and peptone could be utilized as sources of nitrogen. It has also been necessary to revise the opinions that were at first held regarding the secretion of sulphur. The last-named investigators, working with material from the salt marshes of the Crimea, from the neighbourhood of Odessa, and from the Black Sea, found that the secretion of sulphur was not a necessary accompaniment of the activities of these bacteria. This was confirmed by Trautwein who divided them into two classes according to their capacity or incapacity for secreting free sulphur.*

Probably the chemical activities of these bacteria are not as simple as they are represented in the equations formulated by Beijerinck, and this is particularly true of those members that do not secrete sulphur.

Trautwein has shown that they are capable of considerable adaptation, for he has isolated an organism of this class with denitrifying powers. It reduces nitrates to nitrites and nitrogen. Further, the ferments trypsin, lipase, catalase, diastase and oxidase have been identified in its cultures. With extended investigations the number of bacteria belonging to this group has been increased. In addition to the two already mentioned three species were isolated by Issatchenko and Salismowskaja, and were named

Thionsäure-bacterium Beijerinckii,
,, *Nathansohnii*,
,, *Beijerinckii f. Jacobsenii*.

*See page 226 on the nomenclature of these bacteria.

These are regarded as related to, but not identical with, *Thiobacillus thioparus*. Another is that isolated by Waksman and Joffe and named *Thiobacillus thiooxidans*. This organism has the following characters: It is rod-shaped and very small, being less than 1μ long and about 0·5μ in thickness, non-motile, Gram positive, aerobic. It grows readily on solid media, and oxidizes sulphur to sulphates. Carbon is obtained from the CO_2 of the atmosphere, whilst nitrogen is best supplied by ammonium compounds. Growth is stimulated by the addition of such organic compounds as glycerol, alcohol, mannitol, and glucose, and certain inorganic compounds like thallium nitrate, aluminium sulphate and manganese sulphate. The optimum temperature is 28°-30°C. The culture medium is acidified by its development, and the amount of thiosulphate increases steadily with the growth of this species. It is stated that this organism does not cease its development until the acidity reaches the low value of *p*H 0·6—1·0. This is probably the most acid condition under which any microorganism has been known to exist.

H. D. Brown has obtained from sewage and activated sludge an organism which appears to be identical with *Thiobacillus thiooxidans*, and Waksman has recommended a medium in which its cultivation may be best secured:—

$Na_2S_2O_3$, .	5 grams.
KH_2PO_4, .	3 ,,
NH_4Cl, . .	1 ,,
$MgCl_2$, . .	1 ,,
$CaCl_2$, . .	25 ,,
Agar, . .	20 ,,
Distilled water,	1000 c.c.

The physiology of this organism has been investigated by Starkey, who finds that a parallel may be drawn between the rapidity of growth and the extent to which sulphur oxidation has been accomplished. He therefore argues that the oxidation of sulphur is an essential process in the metabolism of *Thiobacillus thiooxidans*. Starkey also found that whilst

the addition of 3 per cent. $Na_2S_2O_3$ induces vigorous growth, 10 per cent. of this substance in a culture medium is inhibitory. Still another member has been isolated which is closely associated with alkali deposits, and which is particularly abundant in the sandy loam soils of the "black" type. This has been provisionally named *Thiobacillus B.* It oxidizes the thiosulphate with liberation of sulphur, and the latter is then oxidized to the sulphate. Its growth is best in a medium with a *p*H value of 6—10.

The activity of the thionic acid bacteria in furthering changes of economic and biological importance in the soil is considerable. It was estimated by Ames that 50 per cent. of the sulphur combined in soil to the extent of 0·5 gram sulphur per 500 grams of soil was changed into the sulphate when the soil was inoculated with these organisms, and that even as much as 70 per cent. was possible if the amount of sulphur was present to the extent of 2·0 grams per 500 grams of soil. As the higher green plants assimilate sulphur in the form of sulphates, the agricultural importance of these organisms is obvious. They are also important in another aspect, for the greater acidity in the soil which follows their multiplication enables the higher green plants to absorb a larger amount of the sulphate, and a greater variety of soil constituents, than is possible in a less acid medium. Many salts which would otherwise be insoluble are thus made available to crop plants. There is, however, a limit to the acidity beyond which harm rather than good would be achieved.

The two organisms *Bacterium crystalliferum* (Gicklhorn) and *Bacterium retiformans* (Gicklhorn) must also be assigned to this group. They were isolated from garden soil that had been covered with water containing potassium sulphide in solution. After three weeks the surface of the water was covered with snow-white points, each being a colony of one or other of these organisms. Between the individual colonies were numerous minute particles of sulphur, evidently derived from the sulphide by oxidation.

Bacterium crystalliferum is 1—2μ long and 0·3—0·5μ broad, and is non-motile. The individuals are closely pressed to-

gether and covered with a very thin layer of slime. Pure cultures were not obtained.

Bacterium retiformans is 2—4·5μ long and 0·5—1·0μ broad. It develops as zoogloea colonies adhering to the walls of the culture flask. It is doubtful whether these two organisms belong to the genus *Bacillus* (or *Bacterium*).

Pseudomonas bipunctatus (Gicklhorn) is provisionally placed in this group, but it is doubtful whether this organism should be classed in the group of Bacteria. Its characteristics are rather those of the simpler Flagellates. Furthermore, its physiology differs somewhat from that of the thionic acid bacteria. It is composed of a single colourless transparent cell, which becomes ovoid during division. Its length is 8—12μ and breadth 3—5μ. It possesses a single, delicate polar cilium, about 12μ long, which propels the organism from behind at the rate of 600μ per minute. Each cell contains two, sometimes three, highly refractive drops. These resemble the large drops found in *Microspira vacillans*. Their composition has not yet been ascertained. They are not composed of sulphur, and the sole justification for the inclusion of the organism in the sulphur bacteria rests on the fact that the organisms show a preference for sulphuretted hydrogen.

After division, many of the daughter cells are temporarily pear shaped, and sometimes even show pointed ends (Fig. 23*c*). Pure cultures have not been obtained.

Habitat.—Found in foul mud from the Botanic Gardens of Graz.

Pseudomonas hyalina (Gicklhorn) resembles the preceding in general form and ciliation, but is smaller, measuring 4—6μ in length and 2—2·5μ in breadth. The reason for its separation from the preceding species lay in the absence of intermediate sizes. As both were found in the same medium, and at the same time, the probabilities are in favour of the two organisms being the same species.

Habitat.—As the preceding species.

Nomenclature of the Thionic Acid Bacteria.

All the members of this group are rod-shaped, and therefore may be included either in the genus *Bacillus*, or the genus *Pseudomonas*. It is unfortunate that Beijerinck should have used the term *Thiobacillus* to designate these organisms, for the prefix *thio* in all morphological classifications is used to designate the bacteria with sulphur contents, and the thionic acid bacteria either do not secrete sulphur, or if they do it is eliminated from the cell. The cumbrous name *Thionsäure-bakterium* (Eng. Thionic acid bacterium) has been suggested and used as a generic title by Issatchenko; and lastly the name *Sulfomonas* has been proposed. In both these changes it would appear as if each innovator had held in view only the needs of his own particular branch of research without reference to the effect which the innovations would have on the efforts of the systematists who have to pass in review all the groups of bacteria. The generic terms *Bacillus* and *Pseudomonas* are sufficiently clearly defined to be used without fear of confusion, and all the known thionic acid bacteria can readily be included in one or other of them. A loose nomenclature based on unstable physiological functions can only lead to ultimate confusion.

The Phylogeny of the Sulphur Bacteria.

The most primitive members of the group are contained in the genus *Lankesteron*, and of these *Lankesteron roseo-persicina* is probably the most primitive. The majority of the sulphur bacteria are probably derived from some such form. This species reproduces by simple fission, and has not an external plasmatic membrane differentiated from the rest of the cell, and in addition it is highly pleomorphic. The more various the number of pleomorphic phases in an organism, the greater its chances of success in a changing environment. Probably the first stage in the advance of such an organism would be the assumption of a stable form under a new set of conditions. If the new conditions persisted for a sufficiently lengthened period a morphologically stable organism could evolve, which

in its turn could serve as a starting-point for a further advance.

From the ease with which it is possible to effect slight morphological and physiological changes in bacterial species it may be argued that the rate of advance of bacterial organisms, and probably of all lowly organisms, is greater than in more complicated forms. Indeed, it is not beyond possibility that some of the organisms described in the early days of Bacteriology may have completely disappeared, and their places taken by species that were not in existence at that time.

The following factors appear to be those most concerned in the evolution of the group:—

A. *Slime Formation.*—In varying degree all the sulphur bacteria form slime from the outermost layers of the cell. In *Beggiatoa alba*, even in motile individuals, a thin layer of slime is always present. Under unfavourable conditions of growth its amount may be considerable.

In *Thiothrix*, on the other hand, slime development is normal, and begins at an early stage in its growth. As the slime subsequently hardens, and as the cells enclosed by it continue their growth before the slime has completely hardened, the structure peculiar to this organism is developed. The filament continues to grow inside a hollow sheath, and its fission is limited to the apical parts that have emerged from the sheath. The fissured fragments when short may be regarded as the precursors of the conidia of more highly developed plants. It is of interest to note that a similar line of development appears to have been followed in the related Iron Bacteria in such organisms as *Cladothrix dichotoma* and *Crenothrix polyspora*.

The zoogloea condition is also in some phases a characteristic of *Lankesteron roseo-persicina*. If this condition is one that is peculiarly favourable to a new environment, a condition temporary under normal conditions may well have become permanent. This probably occurred in the evolution of such organisms as *Thiocystis violacea* in which the cocci are embedded in a mass of slime of a permanent character. The cocci escape in this organism at a definite point in the slime.

In *Thiobacillus Bovistus* a further step is marked by a slightly greater interdependence of the enclosed cells. In *Thiobacillus thiogenus* the advance has taken the form of a more complex covering of slime, an inner and an outer layer being distinguishable, the outer with an obviously protective function. An interesting extension of this line of development is that shown in *Thioploca*, for here the movements of the filaments inside the slimy colony are not unrelated, showing that the units have already lost their independent character. This is the last stage in the evolutionary series along this line.

B. *Enlargement of the Coccus.*—Large cocci are occasionally formed as pleomorphic phases of *Lankesteron roseo-persicina*, and it is possible that the evolution of such organisms as *Chromatium*, *Achromatium*, and *Thioporphyra* may have resulted from the stabilization of similar large forms. Under unfavourable conditions both *Chromatium* and *Thioporphyra* tend to revert to smaller cocci.

C. *Shortening of the Filament.*—The greater freedom of movement possible to short rods in which cilia have developed indicates another line of development. The filaments of such primitive forms as *Lankesteron roseo-persicina* readily break up into shorter lengths; these are probably the precursors of such bacteria as *Thiobacillus* and *Thiopseudomonas*. *Thiobacillus Bovistus* appears to be an intermediate species in this line of development, for in it there is considerable slime formation, whilst the cells on the other hand have attained a certain freedom of movement.

D. *Colonial Habit.*—In *Thiopedia* the cocci, although somewhat loosely arranged, develop symmetrically. In *Rhodothiosarcina* a further advance is noted, as the cocci are closely associated in regular formation, and whilst in *Thiopedia* slime formation is regular, in the other it has become entirely suppressed. The ease with which the disruption of the units of a Sarcina can be brought about so that they exist as uni- and diplo-cocci shows that the grouping of the cells to form colonies is not a racial habit of long duration as time is reckoned in evolution.

E. *Colour*.—The following facts are important in estimating the phylogenetic importance of colour:—

(1) There is little, if any, to choose in point of morphological complexity between the coloured and the uncoloured sulphur bacteria.

(2) The occurrence of colour is not correlated with the possession of any other feature either in structure or in life-history.

(3) There is no distinction in the quality of the pigment between the most primitive and the more highly developed members of the group.

It may be concluded from the third statement that the coloured sulphur bacteria were just as highly coloured on their emergence from still more primitive conditions. Hence we must conclude either that the uncoloured forms were once coloured, and have lost their pigments, or that the sulphur bacteria are of polyphyletic origin. As there are very strong grounds for the belief that photosynthesis occurs in the coloured sulphur bacteria a polyphyletic origin is very probable. There is nothing in common between the two groups apart from what is common to all the microorganisms classed under the Schizophytes, except a common facility for oxidizing hydrogen sulphide to elementary sulphur. In view of the advantage which would be derived from photosynthesis, it is not probable that organisms that flourish in the full light of day, as do the uncoloured sulphur bacteria, would abandon such an obvious advantage. It is much more probable that their ways have lain apart from very early beginnings, and that the uncoloured sulphur bacteria have been derived from still more primitive uncoloured forms. Issatchenko (4), who accepts the photosynthetic view of the coloured sulphur bacteria, in consequence regards the origin of the group as polyphyletic. He states that coloured bacteria like *Lamprocystis roseo-persicina* thrive in a purely mineral solution, drawing their carbon from the carbon dioxide of the atmosphere, and considers that this is possible only to organisms that are capable of photosynthesis.

When the mode of life and the general structure of

Lamprocystis is compared with those of an organism such as *Beggiatoa alba*, which lives in a medium rich in organic matter, the contrast is so great that it is scarcely possible to imagine two organisms at this phase of existence that are so diametrically opposed.

The general conclusion is drawn that the coloured and the uncoloured forms began their present stage of existence as coloured, and as uncoloured organisms, respectively, and that in their further development colour has played no part.

F. *Reproduction.*—In all the sulphur bacteria which have been investigated the prevailing method of reproduction is a

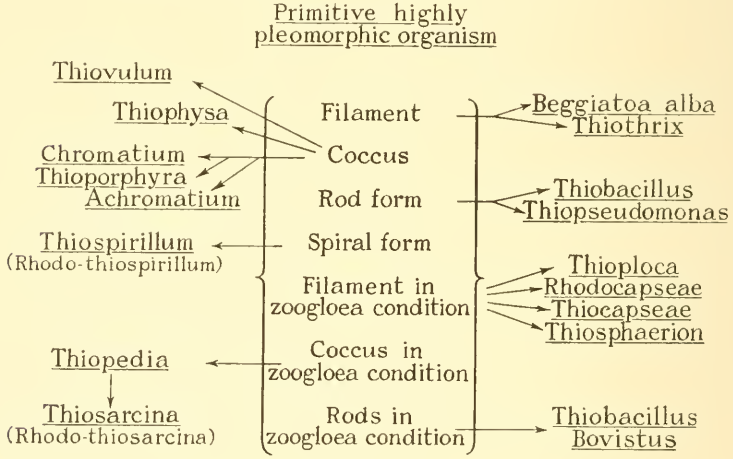

Fig. 64.

simple fission followed by the growth of the separated portions. This method is followed even in such organisms as *Beggiatoa alba*, in which the cell has a slightly differentiated peripheral layer. In *Beggiatoa mirabilis* the formation of a new cell is preceded by the development of a transverse cell membrane as in the higher plants. This, however, is not associated with any other complexity in *Beggiatoa mirabilis*, notwithstanding its relatively gigantic size.

The occurrence of structures that may be endospores in *Beggiatoa alba* and in *Thioporphyra volutans* is at present too problematical for discussion.

The bud formation of *Thioporphyra volutans* appears to be not an advance in methods of reproduction, but rather the recurrence of what is probably a more primitive method of reproduction, for the products of multiplication are of a lower type. Support is given to this view by the fact that it occurs under unfavourable circumstances. The formation of zoospores in *Achromatium oxaliferum* marks a decided advance, but much stress cannot be laid on the fact because it is found only in one organism, and this, one which in its general structure is widely different from the other sulphur bacteria.

These suggested lines of development are schematically represented (Fig. 64).

Summary.

The coloured sulphur bacteria have probably all arisen from the development along several lines of one (or a few) highly pleomorphic primitive organism of a type which is represented among modern forms by such organisms as *Lankesteron roseo-persicina.* It is conjectured that with changes of environment certain pleomorphic forms became stabilized on account of their greater fitness for the changed environment. The uncoloured sulphur bacteria have probably sprung from similar types that had no colouring matter. The sulphur bacteria as a whole are polyphyletic in origin.

The following lines are suggested as having resulted in the modern forms :—

1. Development of hardened slime at an early stage in development, resulting in a hardened sheath surrounding the organism.

2. Fixation of the zoogloea condition with the consequent retention of the units within the slime.

3. The enlargement of the coccus with freedom from excessive slime formation, and greater freedom of action.

4. The contraction of the filament to a length suitable for free and active movement.

CHAPTER XIII.

THE COLOURING MATTER OF THE SULPHUR BACTERIA.

Introduction.

All the chromoparous sulphur bacteria are purple, and range from the pink-purple colour of *Chromatium* to the plum-purple colour of well-developed cultures of *Thioporphyra volutans*. The same culture does not invariably retain the same shade of colour, but frequently passes from one extreme of purple to the other. These shades are due to varying proportions of a purple pigment, named *bacteriopurpurin*, and a yellow-green pigment, *bacteriochlorin*. Again, these two pigments are compound, not simple substances. Their chemical composition has not yet been determined.

Historical.

Ray Lankester (1—2) was the first to investigate the colouring matter of the purple bacteria, which he named *bacteriopurpurin*. This substance was stated to be insoluble in water, alcohol, ammonia, acetic acid, and sulphuric acid. In one place the pigment is said to be insoluble in chloroform, and in another that this reagent changes it to an orange-brown colour, which gradually dissolves. In point of fact the pigment is soluble in chloroform in the dry state, insoluble in the wet state. It is also soluble in alcohol.

Ray Lankester records the following results of his spectroscopic analysis :—

1. End absorption in violet.

2. Two absorption bands, one near the D line, and the other near the E line (see Fig. 65, I).

Interest in the subject was quickened by Engelmann's

discovery that certain purple bacteria, particularly *Bacterium photometricum*, when exposed to light congregated more densely at that part of the spectrum where the absorption of light was greatest (see Chap. XI). He recorded two absorption bands :—

1. 61λ—57λ (orange to green) ; sharply defined
2. 55λ—52λ (green) ; less well defined

} Fig. 65, II.

A darkening was observed in the violet end, beginning near 50λ, and a slight darkening in certain other parts of the spectrum. The maximum light intensity lay in the red at 62λ—63λ. It is thus seen that this pigment differs spectroscopically from the chlorophylls and the carotinoids of higher plants.

Bütschli first observed that there were two pigments, one soluble in alcohol, and the other only slightly so. The former, which imparted a purple colour to the alcohol, formed crystals which turned blue with sulphuric acid, and blue-green with iodine. This occurs also with the colouring matter of *Eugenia sanguinea*. He therefore regarded the colouring matter of both of these organisms as being a form of carotin.

Arzichowsky claimed to have discovered two red colouring matters, which he named *bacteriopurpurin* and *bacterioerythrin* respectively. Unfortunately the first of these names had been used in a different sense by Ray Lankester. His claim for the presence of two colours rested on the fact that a wet filter-paper dipped in the purple extract first took up a rose-red colour, which was a different tint from that left behind. Further, the separation could be effected by shaking the extract with carbon bisulphide when the bacterioerythrin separates from the alcohol, imparting a raspberry colour to the solvent. Molisch (3) experimented with pure cultures of *Rhodobacillus palustris*. He also found two colouring matters :—

1. A green colour which he named *bacteriochlorin*.
2. A red colour which he named *bacteriopurpurin*.

The name *bacteriopurpurin* is used here by Molisch with still another meaning.

Bacteriochlorin is soluble in benzene, olive oil, turpentine oil, and chloroform.

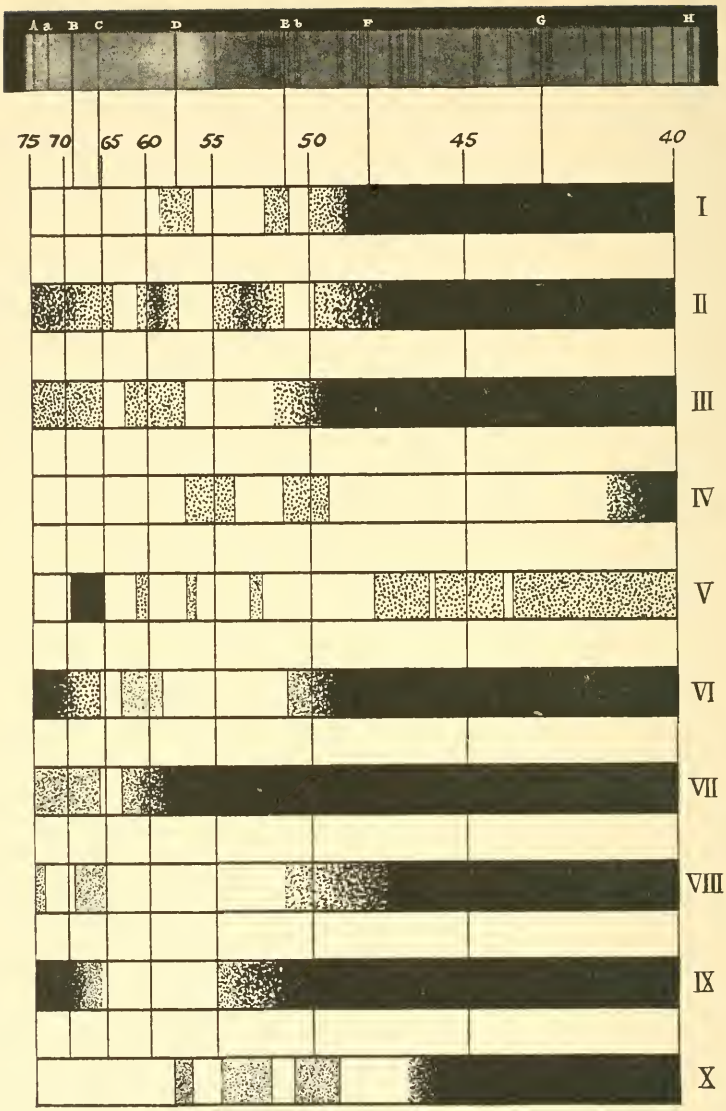

FIG. 65.

Absorption Spectra of the Sulphur Bacteria.

I. The *bacteriopurpurin* of Ray Lankester.
II. Absorption Bands (after Engelmann).
III. *Bacteriochlorin* in absolute alcohol (after Molisch).
IV. *Bacteriopurpurin* α in chloroform (after Molisch).
V. Chlorophyll.
VI.-VIII. *Thioporphyra volutans.*—Three spectra obtained on different days from the same carbon bisulphide extract.
IX. *Thioporphyra volutans.*—Spectrum of coloured extract, taken from culture on cardboard.
X. Phycoerythrin.

SPECTRA.

Bacteriochlorin.—Solution in absolute alcohol:

With coloured fluid 4 mm. thick:
- Absorption at red end from 65λ.
- ,, ,, violet ,, 52·5λ.
- Dark band, 61λ–57λ.

With coloured fluid 10 mm. thick:
- Absorption at red end from 65λ
- Absorption at violet end from 53·5λ
- Dark band 61·5λ–56·5λ

} Fig. 65, III.

Bacteriopurpurin.—Molisch distinguishes two purple pigments:

Bacteriopurpurin α.—Solution in chloroform:

With coloured fluid 10 mm. thick:
- Band I. 56λ–53λ
- Band II. 52λ–48·5λ

} Fig. 65, IV.

Bacteriopurpurin β.—Solution in carbon bisulphide:

With coloured fluid 8 mm. thick:
- Band I. 56λ–53·5λ.
- Band II. 51λ–49λ.

Solution in chloroform:

With coloured fluid 2 mm. thick:
- Band I. 53λ–51λ.
- Band II. 50λ–48λ.

With concentrated solutions Molisch found what appeared to be a third band, but he was not certain of the fact.

He obtained crystals of bacteriopurpurin with the following properties:—

1. Insoluble in water and in glycerine.
2. Slightly soluble in cold absolute alcohol.
3. Insoluble in cold, soluble in hot, glacial acetic acid.
4. Blue to violet colour in pure sulphuric acid.
5. Blue in concentrated nitric acid.
6. Dirty green in iodine and potassium iodide solution.
7. First blue then colourless in strong bromine water.

From these facts he concludes that bacteriopurpurin, as defined by him, is identical with carotin, using this term in a general sense to cover all the carotinoid pigments.

Methods of Investigation.

1. A sample of pond water containing purple bacteria in sufficient numbers to colour the water may be used. Light is passed through a thin layer of fluid, and examined in the spectroscope. This was the method of the earlier investigators, for example, Lankester and Engelmann. The obvious disadvantage of the method consists in the impurity of the fluid. Green Algæ are frequently present and even mere traces of chlorophyll affect absorption spectra.

2. The colouring matter may be extracted by solvents, and the absorption spectra of the coloured fluids examined. This method has the same disadvantage as the first.

3. The most reliable results are obtained when the colouring matter is extracted from pure cultures of the organisms. Only absorption bands of the non-sulphur purple bacteria have hitherto been examined by the third method, because until quite recently pure cultures of the sulphur bacteria have not been obtained. This method, however, is not free from objections, which are those that are inherent in all spectroscopic methods of examination. The chief objection is the extreme unreliability of the evidence obtained in this way when applied to the investigation of complex organic matters. Such evidence must be limited to very wide generalisation, for the following reasons:—

(i) Increase in the concentration of a fluid giving absorption bands leads to a widening of the bands, not to an intensification of their opacity.

The range of wave-lengths occupied by the bands of strong solutions may be considerably greater than those occupied by the bands of weaker solutions, and may extend to several colours of the spectrum. The difference in the range may be so great that whilst four or five different colours are affected by the bands of strong solutions, only two, or at most three, are affected by those of the weaker solutions. It is therefore not possible to fix a definite range of wave-length as characteristic of the bands of any particular coloured fluid.

(ii) This difficulty would not be so great if it were always

possible to fix the same middle point for the bands both of weak and of strong solutions of the same fluid. This is, however, not the case, for the absorption bands change their position even with changes in the specific gravity of the solvent, as was pointed out by Kraus.

(iii) The number and intensity of the bands vary with the solvent.

The Colouring Matter of *Thioporphyra volutans*.

The cultures vary from a pinkish-purple to a deep violet-purple. The same culture frequently shows a variation of colour during its periods of active multiplication. Like other coloured sulphur bacteria it is affected by the intensity of the light to which it is exposed. Bright light is deleterious to its pigment as it is to chlorophyll. On the other hand, during periods when the intensity of daylight is low, as in the winter season in north temperate climates, the colour is not developed. It begins to form in the early summer and persists until late autumn. This seasonal appearance is doubtless correlated with such factors as temperature, presence of dissolved organic matter, etc., which determine the growth of the organism. The absence of colour is not due to the absence of colouring matter, but to the absence of the organism in an active state of multiplication.

Spectroscopic Examination of the Colouring Matter of *Thioporphyra volutans*.

By the use of different solvents two coloured substances were separated:—

1. A purple colouring matter, soluble in chloroform and in carbon bisulphide.

2. A brown or greenish-brown colouring matter, insoluble in chloroform and in carbon bisulphide, but soluble in alcohol and readily soluble in petroleum ether.

The purple colouring matter is unstable, and oxidizes in a few days to a brownish-purple colour.

When the alcohol extract is shaken with petroleum ether a yellowish substance separates from the alcohol, and tinges

the ether with a yellow colour. Hence the alcohol extract contains a component which is more soluble in petroleum ether than in alcohol. The following experiment shows that the bacteriopurpurin obtained by Molisch from the non-sulphur purple bacteria is identical with the purple colouring matter of the sulphur bacteria.

When the purple chloroform extract of *Thioporphyra volutans* was filtered and evaporated, coloured crystals and an oily substance separated out. The crystals were then extracted with cold water, filtered and again evaporated. The residue was a thin brown skin which formed an intense reddish-brown solution with carbon bisulphide. The crystals were similar to those obtained by Molisch. The experiment also showed that in the plant the purple colouring matter is in all probability dissolved in the oil, from which it cannot be extracted by water, but that with the removal of the oil they can be taken up by water.

The spectroscopic examination of coloured extracts from material growing on seaweed was unsatisfactory, for the disintegrated Fucus cells were a source of chlorophylls and carotinoid pigments. The spectrum of the purple fluid exhibited the characteristic bands of chlorophyll; and this occurred even when, owing to careful scraping, at least 90 per cent. of the colour was derived from the microbe.

The organisms were then grown on cardboard in culture pools and the coloured material was collected from the deposit which formed thereon. The absorption spectrum showed a complete absence of absorption bands and localized darkened areas, and the same results were obtained with both the purple and the brown-green fluids. The complete absence of chlorophyll was shown by the absence of the very distinctive dark band in the red between B and C (see Fig. 65, V). There was a certain amount of end-absorption, particularly on the violet side, when the purple fluids were used, and the extent of the end-absorption was dependent on the thickness of the fluid.

This unexpected result appears to place the colouring matter of *Thioporphyra volutans* in a different category from those of the other sulphur bacteria, but it is considered that in

spite of these spectroscopic differences the colouring matter of this microbe is not essentially different from that of the other sulphur bacteria. Its similarity is shown by its behaviour to the various solvents, and by the shape and colour of the crystals formed from the purple extract.

To the doubts that have been cast on the value of absorption spectra as a mode of investigation, the following must be added. When the same purple fluid, containing a preponderating percentage of colouring matter from *Thioporphyra*, was daily examined, the spectrum gradually changed, so much so, that quite a different spectrum was presented at the end of a week. This might be attributed to chemical changes during the period, although such were not apparent, but when the same preparation was again made from the same material and using the same solvents there was frequently no similarity in the absorption spectra. (Compare VI, VII, VIII, Fig. 65.) When cultivated on cardboard the absorption spectrum shown in Fig. 65, IX, was obtained. In this there is nothing to observe except a general darkening on both sides, which is more pronounced on the violet end. The spectra obtained by cultivation on cardboard differed from those obtained by cultivation on Fucus. These spectra, however, showed that in the colouring matter of *Thioporphyra volutans* both chlorphyll and phycoerythrin (Fig. 65, X) are absent.

Spectro-photometric Method of Examination.

By the use of the apparatus described below it is possible to measure the amount of light absorbed at any desired wave-length. Two spectra, one above the other, are viewed at the same time by an arrangement which may be understood by reference to the accompanying diagram (Fig. 66). The light in one passes through the solution, in the other it does not. By means of a cap the whole light can be cut off except for that which comes through a small vertical slit with which the cap is fitted, and which exposes a small area of each spectrum. As the cap can be made to travel over the whole length of the spectrum a comparison can be made of the intensity of the light from the two spectra at any selected point.

The light from L_1 passes through a glass screen (A_1), the

prism (R), the collimator (C), and the prism (P), by which it is refracted and passes into the telescope (T). It enters the lower part of the slit at S and forms the upper spectrum. The light from L_2 passing through a glass screen at A_2 is reflected by the prism R and enters the upper part of the slit and forms the lower spectrum. In comparison with the refracted light the reflected light loses 8 per cent. of its intensity, which must be allowed for in the calculation. In order to produce spectra of the same intensity, the light L_2 is kept fixed and the light L_1 moved backwards or forwards until both spectra appear to be precisely similar.

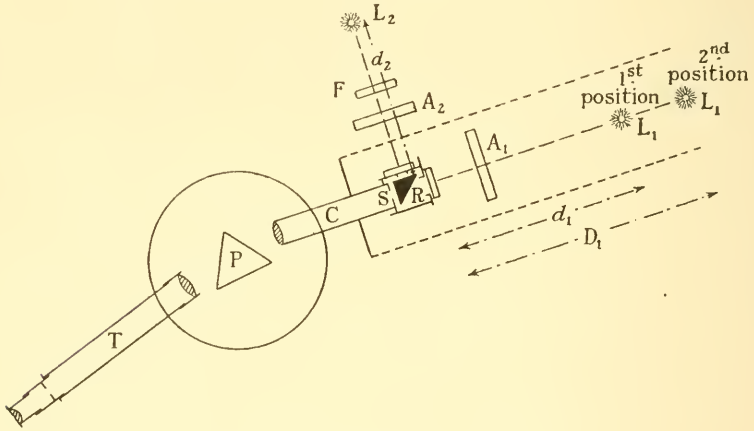

FIG. 66.

Let I_1 and I_2 be the respective intensities of L_1 and L_2. Then if d_1 and d_2 be their respective distances when similar spectra are produced, we have

$$\frac{I_1}{(d_1)^2} = \frac{I_2}{(d_2)^2} \cdot \frac{92}{100}. \quad . \quad . \quad . \quad (1)$$

The coloured fluid is now placed in position (F), and reduces the intensity of the lower spectrum. Next the light L_1 is moved backwards to some position at a distance D_1 at which the two spectra are once more of the same intensity.

The insertion of the coloured fluid alters the intensity of the light from L_2. Let its new value be I_2'.

$$\therefore \frac{I_2'}{(d_2)^2} \cdot \frac{92}{100} = \frac{I_1}{(D_1)^2}. \quad . \quad . \quad . \quad (2)$$

From equations (1) and (2) we get

$$\frac{(I_2')}{I_2} = \frac{(d_1)^2}{(D_1)^2}. \quad . \quad . \quad . \quad (3)$$

Hence

$$I_2 : I_2' = 1 : \frac{(d_1)^2}{(D_1)^2}.$$

Or the fraction of the light absorbed by the coloured fluid is thus

$$1 - \frac{(d_1)^2}{(D_1)^2}.$$

Thus the effect of the insertion of the coloured fluid on the intensity of the light can be accurately calculated for each part of the spectrum.

Results with *Thioporphyra volutans*.

Confirmation was obtained of the absence of definite absorption bands and localized darkened areas in the absorption spectra of this organism.

Summary.

The colouring matter of *Thioporphyra volutans* is similar to that of other sulphur bacteria in being composed of two groups of substances, one of which forms a purple colour in some solvents, *e.g.* chloroform, whilst the other forms a brownish-green colour in other solvents, as *e.g.* ether. The former does not appear to be identical with the phycoerythrin of the Florideæ (Fig. 65, X), nor does the latter show any relationship with chlorophyll. Spectroscopic analysis show the absence of absorption bands and of localized darkened areas in cultures on cardboard, but these were found in cultures on Fucus. The want of stability of such bands and areas in the absorption spectra makes this method of investigation very uncertain. At present it is not possible to associate the colouring matter of *Thioporphyra volutans* with any arrangement of dark bands in its absorption spectrum.

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